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PATHOLOGY

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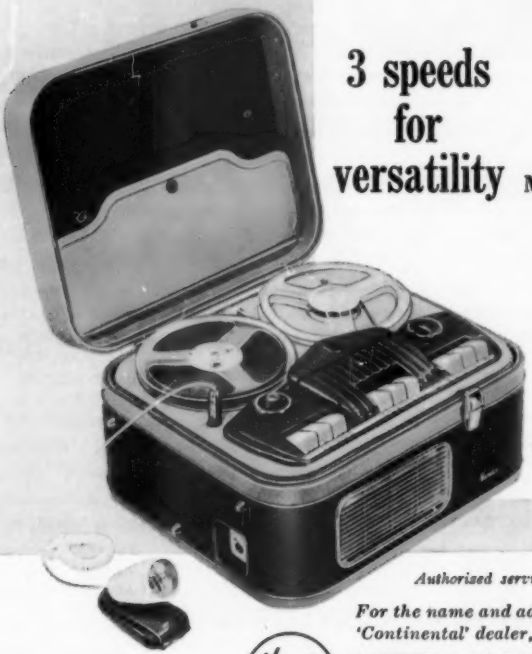

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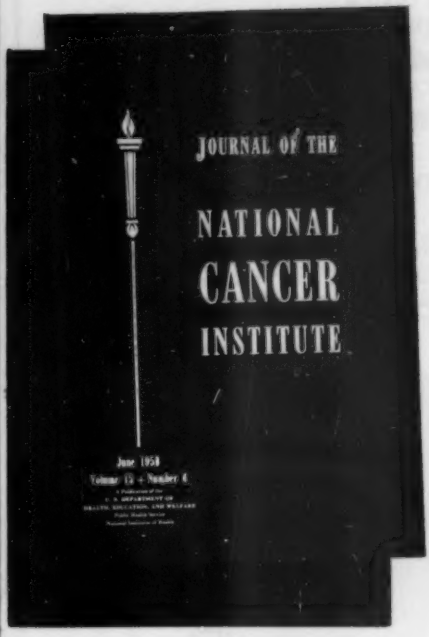

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Studies on the Chemical Pathology of Lesions Produced by Ethionine

EMMANUEL FARBER, M.D., Ph.D., New Orleans

The structural changes in cells which pathologists have come to recognize in disease are merely visible manifestations of altered cellular chemistry, metabolism, and organization at a molecular level. The pathogenesis of many morphologic alterations can therefore never be fully understood until we can define the underlying chemical disturbances. This field of chemical pathology has long interested pathologists, but satisfactory techniques for its study have only recently been made available through advances in cellular biochemistry.

A concept very useful in the study of chemical pathology is that of metabolic antagonism. This is an old idea, but it is only recently that it has been placed on a solid experimental basis.[1,2] As you well know, this notion states that one can, so to speak, fool the living organism by presenting it with a compound which differs only slightly from a naturally occurring metabolite. The artificial agent is capable of reacting with the enzyme systems of the cell in such a way as either to inhibit the utilization of a normal substrate or to replace the normal substrate in the cell metabolism. The concept of metabolic antagonism has furnished the basis for much modern re-

Fig. 1.—Emory Warner, M.D., Chairman of Pathology, University of Iowa, President of American Society for Experimental Pathology, presented the second annual award of the Society to Emmanuel Farber, M.D., of Tulane University, New Orleans, at the ASEP banquet, in Philadelphia, April 16, 1958. The award is symbolized by a medallion and a $1,000 honorarium contributed by Parke, Davis & Company. Dr. Farber, 39, won the honor for his basic research into chemical changes in cells.

Submitted for publication July 1, 1958.

Departments of Pathology and Biochemistry, Tulane University School of Medicine.

Read before the 1958 meeting of the American Society for Experimental Pathology on the occasion of the second annual Parke-Davis Award in Experimental Pathology.

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search in chemotherapy, general pharmacology, and biochemistry. It offers pathologists an excellent opportunity to study the biochemical basis for some disease processes, since it simplifies the initial search for the possibly significant biochemical derangements in disease states.

The work I should like to discuss this morning is concerned with the attempt to understand some of the metabolic changes in cells preceding and accompanying structural lesions induced by ethionine, a metabolic antagonist of methionine. This artificial compound, which so far has not been reported to occur naturally, was first synthesized by Dyer, in 1938, and differs from methionine only by having the S-methyl group replaced by an S-ethyl.

Fatty Liver

At the time that we began our studies on ethionine, it was generally believed that many kinds of fatty liver in animals and in humans were related to a deficiency of choline and methyl groups. Choline was considered the primary compound, and other compounds, such as methionine, were thought to act only in the secondary role of contributing methyl groups for the formation of choline.[3,4] Such a relationship was demonstrated in experimental animals on low-methionine diets devoid of choline. On a molar basis, choline is most effective in preventing the choline-deficiency fatty liver, while methionine is considerably less effective.[5] An important feature of the fatty liver of choline deficiency is the centrolobular distribution of the excess lipid, as Hartroft demonstrated so beautifully in 1950.[6]

When Drs. Simpson and Tarver and I found that the injection of ethionine rapidly induced a fatty liver,[7] we naturally assumed that we were dealing with a choline-deficiency fatty liver. Ethionine was presumably inhibiting the demethylation of methionine and thereby decreasing the endogenous supply of choline. We were therefore surprised to find that choline administration was ineffective in preventing the ethionine-induced fatty liver.[7,8] We gave choline by stomach tube daily for several days before injecting ethionine; we injected choline; we incorporated excess choline in the diets the animals ate before receiving ethionine, and in every instance choline failed to protect.

In contrast, methionine was highly effective[7,8] and appeared to be specific, since several other amino acids, including cysteine, homocysteine, lysine, glycine, valine, and glutamic acid, gave no protection.[7-9]

We thus appeared to be dealing with a new type of fatty liver related to methionine but not to choline, and subsequent work bore out this assumption. In ethionine-treated animals, the excess fat in the livers was clearly periportal in distribution, contrasting sharply with the centrolobular localization in choline deficiency.[10] The excess lipid incidentally was essentially all neutral fat.[11]

These facts convinced us that methionine, in addition to its ability to help the body synthesize choline, could also act through some different pathway in protecting animals against fatty liver, in this instance a periportal fatty liver. This conclusion has recently received further confirmation from the work of Dr. Sidranskv. He has found that when adult rats are force-fed diets adequate in choline but devoid of methionine they also develop a periportal fatty liver indistinguishable from that seen in ethionine-treated animals.[12]

As part of our initial study, we were interested in finding out what effect ethionine might have on the intermediary metabolism of methionine. One objective was to learn whether ethionine was a true antagonist of methionine, and another was to search for some clue to the pathogenesis of the fatty liver. Dietary methionine has a varied fate. Some is used for protein synthesis, and the remainder is degraded or altered in several different ways. For example, the S-atom may be used for the synthesis of cysteine; the methyl group is

available for transmethylation reactions in the synthesis of several compounds, including choline, or it may be oxidized to formic acid—to name but a few of the known reactions. Some of these reactions were studied by us [13,14] or by Siekevitz and Greenberg,[15] and all were found to be inhibited to a marked degree in the livers of the ethionine-treated animals. In addition, the incorporation of labeled methionine into liver protein was also inhibited in animals receiving ethionine.[13] Interestingly enough, the incorporation of glycine [13] and of several other amino acids was also inhibited,[16] suggesting that ethionine had a general inhibitory effect on protein synthesis in the liver. This effect appeared to be related to some interference with methionine metabolism, since it could be reversed by methionine but not by glycine or valine.

Both the morphologic and the biochemical effects of ethionine on the liver had now been linked to some physiologic or biochemical reaction of methionine, but one could not even suggest what specific biochemical disturbance might be responsible for the development of the fatty liver.

New insight came when we compared the effects of ethionine in males and females. Thus far, female rats had been used in most of the experiments, but in completing an early phase of the work several experiments were done with males. To our amazement, the males did not develop the fatty liver which the females showed so readily.[7,17] Identical results were obtained not only in the Long-Evans strain but subsequently in Wistar, Carworth Farms, Fisher, and Sprague-Dawley rats. Jensen and co-workers have since confirmed these results in Slonaker rats.[11] Recently, Dr. Sidransky has found the same sex difference in the periportal fatty liver induced by diets adequate in choline but devoid of methionine.[12]

We then undertook a further study of the basis for the sex difference in the effects of ethionine. We found that oophorectomy in females or estrogen administration to males had no influence on the occurrence of the fatty liver. In contrast, castration rendered males susceptible to the induction of fatty liver and testosterone propionate protected females.[17] Subsequently, in a joint study with Dr. Segaloff, we treated castrated females with a whole series of preparations, including hormones derived from the anterior pituitary, thyroid, adrenals, gonads, and pancreas. Only androgens and pituitary growth hormone protected against the ethionine-induced fatty liver.[18] We have therefore tentatively concluded that the resistance shown by the males is due, at least in part, to the presence of androgens.

We were, of course, interested in knowing whether other hepatic effects of ethionine would show a similar sex difference. Some effects of ethionine, such as inhibition of the transfer of the methyl group from methionine to choline,[14,19] inhibition of the oxidation of the methionine methyl group to formic acid,[15] and inhibition of choline oxidase,[20] were found by others or by us to show no comparable sex difference.

One biochemical effect, however, did show the same sex difference as the fatty liver. In females, but not in males, ethionine was shown to inhibit protein synthesis in the liver.[16] Initially, we measured protein synthesis by incorporation of radioactive amino acids into liver and plasma protein in vivo. Later, we also measured the labile enzyme system, the tryptophan peroxidase, and the incorporation of radioactive leucine into protein in a cell-free system in vitro. With all three methods, the females showed inhibition and the males did not.

The results with the in vitro incorporation of amino acids into protein are of special interest, and I should like to digress for a few minutes to discuss these results and their implications. In this system, described by Zamecnik and co-workers,[21,22] one homogenizes the liver and, by differential centrifugation, separates the small submicroscopic cellular fraction, the

microsomes, and the final soluble supernatant from the unbroken cells, nuclei, mitrochondria, red cells, and other debris. If one incubates the microsomes plus the supernatant with a radioactive amino acid, a suitable source of energy, and certain co-factors, one can demonstrate the incorporation of the labeled amino acid into protein. The microsomes are actually broken-up pieces of the endoplasmic reticulum, plus the tiny granules frequently associated with the reticulum, as Palade and Siekevitz [23] and Littlefield et al.[24] have shown by electron microscopy. The supernatant fraction contains among other things enzymes which activate amino acids for incorporation and soluble ribonucleic acid which appears to take part in the incorporation process. When microsomes were prepared from the livers of female rats given injections of ethionine a few hours previously, they showed about 75% less incorporation of radioactive leucine than when microsomes from untreated females were used. The activity of the supernatant fraction in promoting amino acid incorporation was not altered by pretreatment of the animal with ethionine, provided female rats were used. In other words, a defect appears to be imposed upon the hepatic microsomes of female rats within five hours after giving them injections of ethionine.

In males, the situation is quite different. Here, the microsomes from the ethionine-treated animals are at most only slightly less active than are those from the controls. However, in this sex, the supernatant shows interesting differences. The supernatant from ethionine-treated males is considerably more active in facilitating incorporation than is that from the control male. The effect is the same regardless of the origin of the microsomes.

We are therefore, for the first time, beginning to localize the ethionine-induced effects to certain well-defined cytoplasmic components. I am sure we would all agree that a full understanding of many pathologic responses awaits the identification of specific biochemical disturbances located in particular cytoplasmic or nuclear components of the affected cells. We have not yet accomplished this with ethionine intoxication, but at least some progress has been made. These same findings, incidentally, open up interesting possibilities for investigating the mechanisms through which androgens and growth hormone influence protein metabolism.

The reproducible association of fatty liver and inhibition of protein synthesis suggests that there may be a casual relationship between these two disturbances in metabolism. Since the protein changes precede the fatty liver by a few hours, the disturbances in protein metabolism may be presumably the primary effect of ethionine, insofar as the fatty liver is concerned. The exact mechanism, if there is one, relating these two disturbances remains to be clarified.

For a number of years, accumulating evidence has pointed with increasing emphasis toward a relationship between protein and amino acid deficiencies and some forms of fatty liver, particularly those in which the fat first appears periportally. Interest in this relationship has been further stimulated by the human disease kwashiorkor. Kwashiorkor is a nutritional disorder of children, common in Africa, India, and several other countries and believed to be due primarily to a dietary deficiency of "high quality" protein containing the proper amounts of all the essential amino acids.[25] Pathologically, periportal fatty liver, pancreatic acinar atrophy, and salivary gland atrophy are among the prominent findings. Since essential amino acid deficiencies are known to interfere with protein synthesis, it is generally believed that many of the pathologic alterations in kwashiorkor may be the result of such an inhibition.

The disturbed hepatic protein metabolism, periportal fatty liver, pancreatic damage,[8,26] and damage to submaxillary and gastric mucosal glands [27] induced by ethio-

nine administration suggested that this agent might be producing a simplified experimental model of the more complex human disease, kwashiorkor. If this is so, then the animal experiments with ethionine could be helpful in clarifying the pathogenesis of the human disorder. In a recently completed study by Dr. Sidransky, many of the pathologic findings seen in kwashiorkor and some of those induced by ethionine were reproduced by force-feeding rats with diets devoid of threonine or histidine.[28,29] However, the biochemical findings did not coincide completely with those found with ethionine. We are therefore not at all certain that the essential cellular alterations in ethionine-treated animals and in the protein- or essential amino acid-deficiency states are similar.

Pancreas

So far, nothing has been said about the effects of ethionine on the pancreas or about the long-term changes in the liver. In Dr. Popper's laboratory, in Chicago, we observed that many rats developed peritoneal fat necrosis and gross and microscopic evidence of pancreatic damage 48 to 72 hours after they had been given injections of ethionine.[8] The animals also showed degenerative changes in the testes, as was subsequently reported by Alvizouri and Warren[30] and Kaufman et al.,[31] as well as degeneration of the epithelium of the proximal convoluted tubule,[7] described in detail by Wachstein and Meisel.[32] Since most of our work was concerned with the pancreas, I shall limit myself to a discussion of this organ.

In agreement with Goldberg et al.,[26] who reported the pancreatic changes simultaneously with us, we found that the first change was a swelling of the acinar cells of the pancreas, along with a striking loss of basophilia. This change occurred in both sexes within about 12 hours after we gave ethionine. By 48 hours, many of the pancreatic acini were undergoing necrosis with a concomitant acute inflammation. Subsequently, as several workers have reported,[26,27,30,33] there occurs a disappearance of the affected acini and replacement by adipose tissue. The islets are preserved and even appear to show some hyperplasia in later stages. As Fitzgerald and Alvizouri[34] and Kinney and co-workers reported,[35] the pancreas regenerates rapidly.

The pancreas, of course, is an organ which produces large quantities of protein enzymes and in which protein synthesis is therefore very active. Since ethionine appeared to inhibit protein synthesis in the liver, it was reasonable to assume that the same biochemical lesion occurring in the pancreas was the biochemical basis for the pancreatic damage.

Dr. Sidransky and I began to study protein synthesis in the pancreas under the influence of ethionine, with the hope of learning something about the role of disturbed protein metabolism in the pathogenesis of pancreatitis. To our great surprise, all our experiments showed that not only does ethionine *not* inhibit protein synthesis in the pancreas but it actually increases or stimulates protein synthesis in both sexes.[36] This work was limited to the first 24 hours after ethionine injection, by which time the acinar swelling is already very pronounced. At this stage, any primary biochemical lesion should be well developed. Methionine administration protected the animals against both the morphologic[8] and the biochemical[36] changes induced by ethionine. Choline and cysteine were both without effect. We have recently found that there appears to be a similar increase in pancreatic protein synthesis in immature rats force-fed a methionine-devoid diet.[29]

On the basis of these results we concluded that the pancreatic lesions were in some manner related to an interference by ethionine with the normal metabolism of methionine but that the primary functional change was certainly not an inhibition of protein synthesis. The recent findings on the effects of ethionine on the in vitro incorporation of radioactive amino acids into

protein in liver fractions from male rats may offer a clue to the mechanism of action of ethionine on protein metabolism in the pancreas.

Liver Cancer

Before concluding, I should like to say a few words about the chronic effects of ethionine upon the liver. This phase of our knowledge of ethionine was initiated by Popper and co-workers,[37] who reported that rats fed diets containing ethionine developed tumor-like nodules in the liver. Because of our interest in ethionine, we repeated this work in an effort to obtain unequivocal carcinoma of the liver. By minor modifications of the diet used by Popper and by decreasing the dietary level of ethionine, we were able to keep almost all our animals alive for 10 months or longer. About 85% of these animals developed large tumor masses in the liver after being on the diets for eight months.[38] Many of the tumors measured up to 6 cm. in diameter and showed evidence of necrosis and hemorrhage. In over half of the animals, metastases were present when they were killed. To date, we have produced cancer of the liver in over 75 rats and have found that the tumors, with a single exception, were hepatocellular carcinomas. Although cholangiofibrosis was a constant lesion, only one poorly differentiated cholangiomatous carcinoma has been observed. It is particularly interesting that the striking sex difference in the inhibition of hepatic protein synthesis and fatty-liver production that characterized the acute experiments was not apparent in these long-term experiments.

The possible protective action of methionine or choline was tested against the chronic effects of ethionine. Choline was especially interesting, since the chronic feeding of diets low in methionine and devoid of choline has been found by Copeland and Salmon,[39,40] Wilson,[41] and Buckley and Hartroft[42] to induce malignant neoplasms of the liver in rats, chickens, and mice. In our experiments, methionine in adequate amounts gave complete protection against all the liver changes induced by ethionine, including liver cancer, while even large amounts of choline offered only partial protection. Betaine was more effective than choline. So far, among the substances we have tested, no other compounds related metabolically to methionine have shown any protective action.[43]

By itself, the finding of still another hepatic carcinogen is of no great interest. However, it is felt that ethionine as a carcinogen has something special to offer. Firstly, ethionine induces a sequence of early histologic changes in the liver preceding the appearance of cancer, which is remarkably similar to that induced by butter yellow and its derivatives, acetylaminofluorene, and several other hepatic carcinogens.[44] Secondly, the fact that the biological effects of ethionine appear to be related to the metabolism of methionine offers a focal point for further studies of the biochemistry of carcinogenesis. Thirdly, some metabolites of methionine, when added to the ethionine-containing diet, have been shown to influence the preneoplastic changes in the liver. For example, when homocystine is fed to rats along with ethionine, the nodular hyperplasia of the liver cells is greatly exaggerated.[45] These animals develop liver nodules which may measure up to 2 cm. in diameter as compared to a maximum size of 0.5 cm. in control animals followed for the same period without homocystine.

This brief review of certain of the biological effects of ethionine has purposely been limited to those aspects of the problem with which I have had some direct experience, and much excellent work in closely related fields has not even been mentioned.

In conclusion, ethionine, a metabolic antagonist of methionine, induces a periportal fatty liver, inhibits some of the known biochemical reactions of methionine in the liver, and also interferes with hepatic protein synthesis. The fatty liver and acute

inhibition of protein synthesis occur only in female rats, not in males. The difference appears to be related to the presence of androgens in the male. Ethionine induces acute pancreatic acinar damage, and when it is added to the diet carcinoma of the liver results. Almost all the known effects of ethionine are readily prevented by the administration of methionine but not by choline or by several other amino acids. The available evidence suggests that methionine may play an important regulatory role in protein metabolism and that at least some of the pathologic effects of ethionine administration result from an interference with this modulating function of methionine. The observations we have reported in this paper are obviously only a beginning in the unraveling of the pathogenesis of a few experimental lesions. Nevertheless, they do illustrate some of the potentialities of a combined biochemical and morphologic approach to the study of disease.

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Relation of Splenic and Lymph Node Changes to Hypergammaglobulinemia in Cirrhosis

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Elevation of serum γ-globulin accompanies hepatic disease,[1] particularly cirrhosis, reaching the highest levels in the postnecrotic variety.[2] The mechanism of this elevation and the site of γ-globulin formation under these circumstances are poorly understood. It has been suggested that hypergammaglobulinemia is related to increased Kupffer-cell[3] or liver-cell[4] activity. However, there is no evidence that the liver forms γ-globulin,[5,6] at least under normal circumstances. Recent observations, based on a comparative study of serum γ-globulin levels and cytoplasmic pyroninophilia, implicate the spleen and abdominal lymph nodes in the γ-globulin elevation in ethionine-induced rat cirrhosis.[7] An extension of this study to human cirrhosis therefore appeared desirable.

Material and Methods

Small pieces of liver, spleen, bone marrow, and lymph nodes were obtained at necropsy of 30 patients with diffuse septal or postnecrotic cirrhosis and 8 apparently healthy persons dying shortly after gunshot wounds.* The lymph nodes were samples of both thoracic (upper mediastinal or cervical) and abdominal (periaortic or mesenteric) nodes. The tissues were fixed in Carnoy's solution, and paraffin sections were stained with hematoxylin and eosin and with methyl green and pyronin. Control sections were stained with methyl green and pyronin after digestion with ribonuclease. In all cases of cirrhosis, preterminal γ-globulin determinations had been performed by turbidimetric estimation.[8]

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* Dr. V. Levine provided this material.

Observations

1. *Types of Cells with Cytoplasmic Pyroninophilia.*—Several types of pyroninophilic cells were encountered in lymph nodes and spleen.

A. Cells contained large vesicular, round, oval, or lobulated nuclei, with several prominent nucleoli and intensely (red) staining granular cytoplasmic rims; these cells resembled primitive reticulum cells.

B. Cells exhibited smaller, round or oval, vesicular nuclei; one or two prominent nucleoli, and cytoplasmic rims with intensely pyroninophilic granules and occasional vacuoles; these cells resembled lymphoblasts.

C. The nucleus, although still vesicular and larger than that of the lymphocyte, showed irregular clumps of chromatin. No distinct nucleoli could be seen. The cytoplasm was more abundant and contained pyroninophilic granules and often vacuoles. The nucleocytoplasmic ratio was approximately 1. These cells were similar to immature lymphocytes.

D. This cell closely resembled Type C except that the chromatin tended to be arranged in clumps along the nuclear margin. This cell resembled an immature plasma cell.

E. The nuclei were smaller, were eccentrically placed, and contained radially ar-

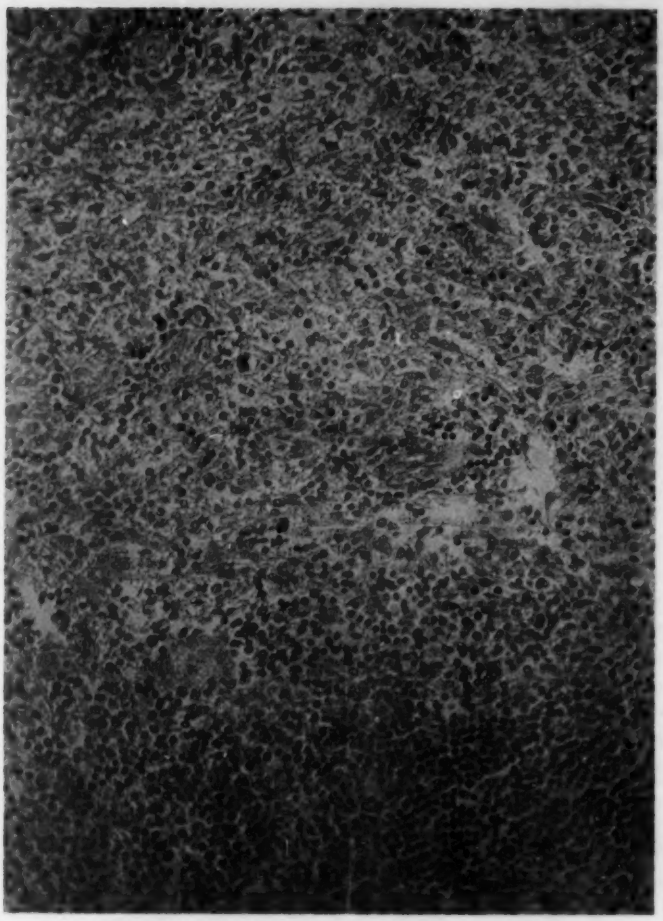

Fig. 1.—Spleen of control subject. Scattered isolated pyronin-staining cells are seen in the red pulp. Methyl green and pyronin stain; × 240.

ranged chromatin clumps. The abundant cytoplasm often exhibited vacuoles, occasionally filling the entire cell. These cells resembled plasma cells. Cells with similar nuclei not associated with pyronin-staining cytoplasm were frequently observed.

F. Relatively small cells with small round nuclei and dense nuclear chromatin and a pale narrow pyroninophilic rim with indistinct margins; these cells resembled lymphocytes.

2. *Distribution of Pyroninophilic Cells in the Spleen and Lymph Nodes of Control Subjects.*—In the spleen (Fig. 1), pyroninophilic cells resembling primitive reticulum cells (Type A) and lymphoblasts (Type B) were found within the Malpighian follicles, while a ring of varying width of Type B cells was found at the periphery of the follicles. Within the red pulp, scattered cells of Types A and B were found in addition to even fewer pyroninophilic cells, resembling mature and immature plasma cells (Types D and E). Type F cells were noted in the periphery of the cortical follicles of the lymph nodes. Few single cells of all types of pyroninophilic cells were encountered in the medullary portion (Fig. 2).

3. *Distribution of Pyroninophilic Cells in Spleen and Lymph Nodes in Cirrhosis.*—Despite individual variations a distinct pattern was apparent. In the spleen the distri-

bution of pyroninophilic cells in the white pulp was similar to that found in the control cases, though they were often present in greater number. The follicles were frequently smaller, especially in the presence of fibrocongestive changes. The red pulp contained considerably more pyroninophilic cells than was seen in control cases (Figs. 3 and 4). These cells were predominantly of Types B, D, and E ("immature lymphocytes," "immature plasma cells," and "mature plasma cells"). Moreover, the pyroninophilic cells exhibited many mitoses and were arranged in groups, often including several cell types and contrasting with adjacent non-pyronin-staining groups of cells. The distribution or the estimated number of pyroninophilic cells was the same in thoracic and abdominal lymph nodes in the same person. Throughout the medullary cords there were groups of pyroninophilic cells resembling those described in the red pulp of the spleen (Figs. 5-7). Pyroninophilic cells were sometimes seen within foci of inflammatory cells in the cirrhotic livers. Such foci were not present in the control series.

4. *Correlation Between Degree of Cytoplasmic Pyroninophilia and γ-Globulin Elevation.*—The cytoplasmic pyroninophilia was graded from 1 to 4+ by two independent observers for correlation with the

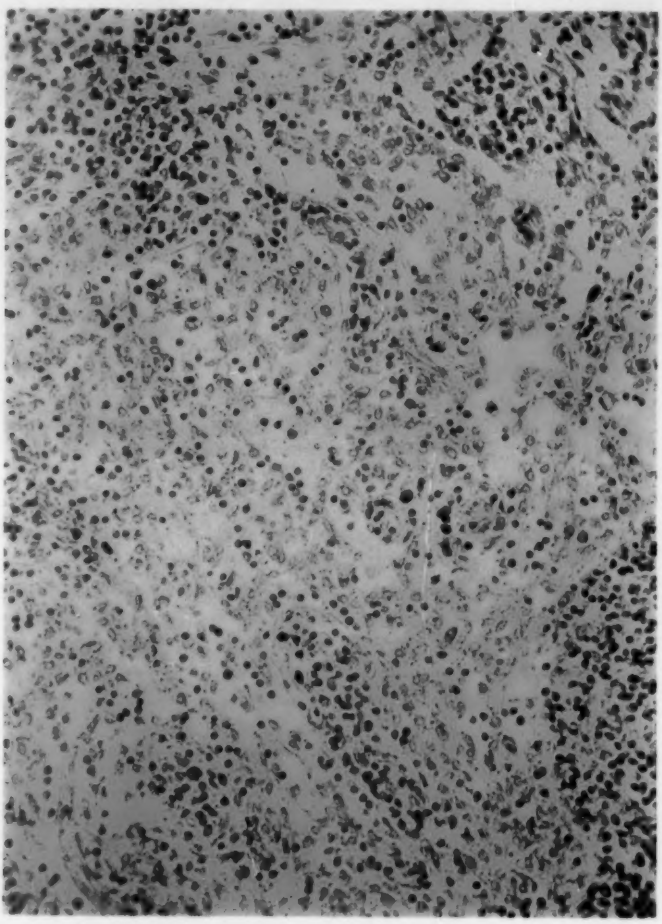

Fig. 2.—Lymph node of control subject, showing isolated pyroninophilic cells in the medullary cords. Methyl green and pyronin stain; × 240.

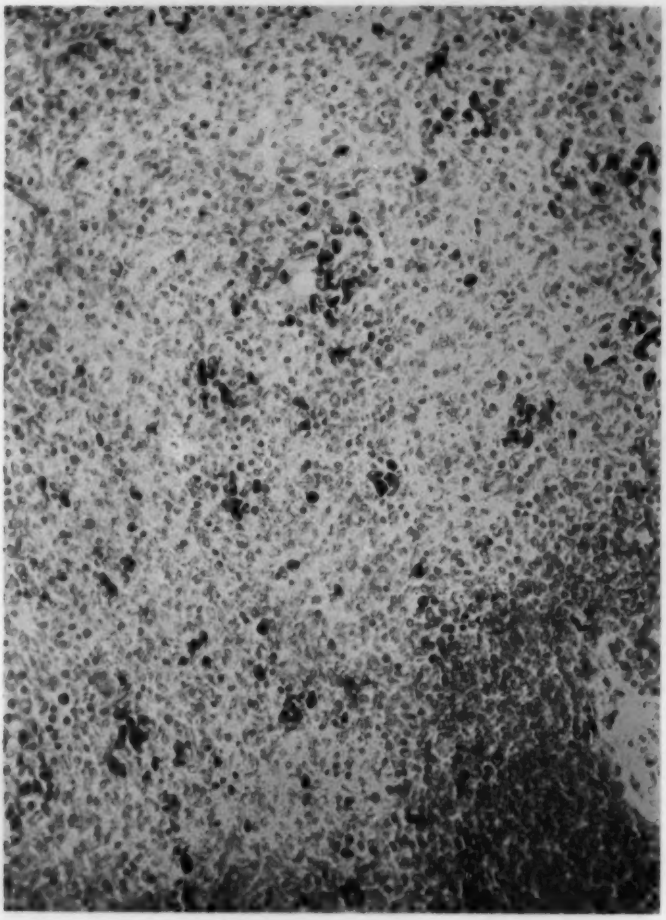

Fig. 3.—Spleen in cirrhosis. Clusters of pyroninophilic cells in the red pulp. Compare with Figure 1. Methyl green and pyronin stain; × 240.

γ-globulin levels. Emphasis was placed upon the red pulp of the spleen and the medullary cords of the lymph nodes, since they presented the striking contrast between cirrhotic and control cases. In the rare instances of discrepancy, an additional number of fields were reviewed. Pyroninophilia in lymph nodes and spleen (Figs. 8 and 9) correlated fairly well with the serum γ-globulin levels, although there were some exceptions. The best correlation was obtained (Fig. 10) when the grading of lymph nodes and spleen was averaged. The excess of pyroninophilia in spleen and lymph nodes ran parallel.

5. *Correlation of Splenic Weight with Pyroninophilia and γ-Globulin Levels.*—No definite correlation could be established between the splenic weight and pyronin-staining cells, though many of the enlarged spleens showed a high degree of pyroninophilia; nor was there a correlation between the weight of the spleens and the γ-globulin levels. The last finding was confirmed by a review of 50 additional autopsy cases.

Comment

The distribution of the various cells with cytoplasmic pyroninophilia in persons with diffuse septal and postnecrotic cirrhosis

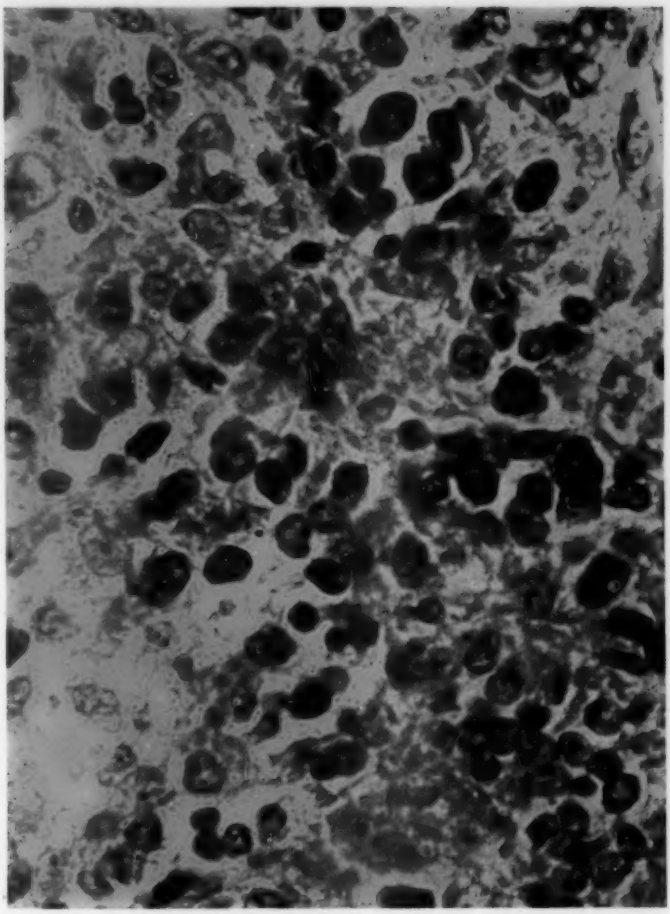

Fig. 4.—Spleen in cirrhosis. Cluster of pyroninophilic cells in the red pulp. Note different cell types. Methyl green and pyronin stain; × 1040.

differs markedly from that in control subjects. In cirrhotic patients the medullary cords of the lymph nodes and the red pulp of the spleen contain prominent clusters of several types of these cells, while only a few scattered pyroninophilic cells are seen in these locations in the control cases.

Pyronin stains, when controlled by ribonuclease, indicate the presence of ribonucleic acid (RNA).[9] There is strong evidence that RNA is related to protein synthesis and that its tissue distribution indicates the site of protein formation.[10-14] Immature cells in general are rich in cytoplasmic pyroninophilia, probably reflecting the active protein formation associated with the growth of the cell. The pyroninophilia of the lymphoid- and plasma-cell series has been found associated with the synthesis of immune globulin [13-18] or excessive γ-globulin.[7] The pyroninophilic cells which proliferate during antibody formation resemble those observed with the hypergammaglobulinemia in rat cirrhosis.[7] The parallelism between the estimated degree of pyroninophilia and the degree of γ-globulin elevation suggests that the excess of serum γ-globulin in human cirrhosis is produced at least in part by the lymph nodes and spleen, in keeping with similar observations in ethionine-induced rat cirrhosis. Furthermore, the comparable pyroninophilia in the spleen and in

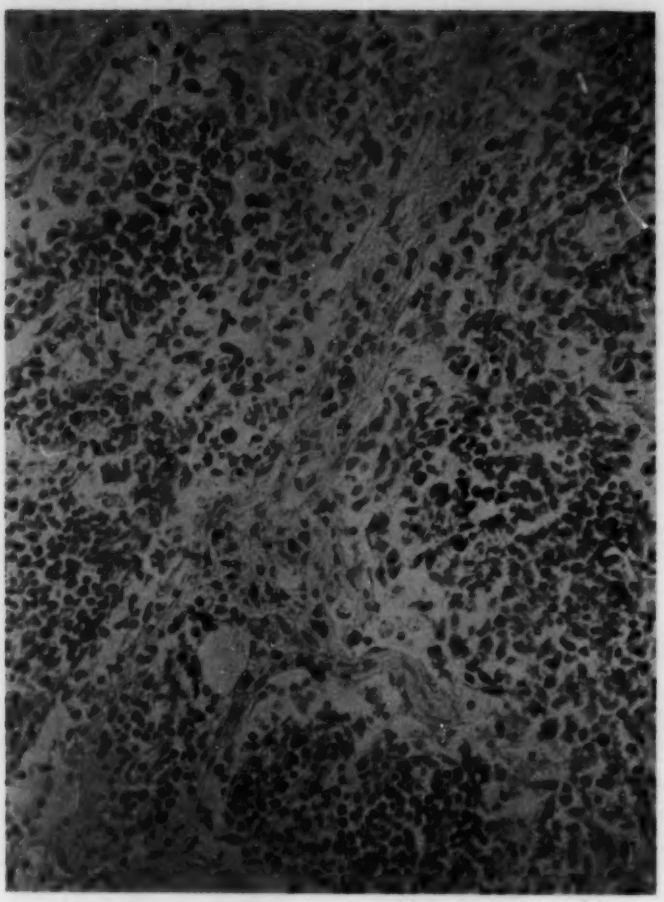

Fig. 5.—Lymph node in cirrhosis, showing clusters of pyroninophilic cells in the medullary cords. Methyl green and pyronin stain; × 240.

abdominal as well as thoracic lymph nodes seems to indicate a generalized stimulus to γ-globulin synthesis.

The nuclear structure of the pyronin-staining cells in the splenic pulp and in the medullary cords of the lymph nodes suggests participation of both lymphoid- and plasma-cell series. This is in contrast to the findings in rat cirrhosis, where mature plasma cells could not be related to the excess γ-globulin formation. The human material also shows considerable numbers of cells appearing as lymphoblasts or primitive reticulum cells. Both plasma cells [19-22] and lymphocytes [23-28] seem to be active during the formation of immune globulin. Participation of both lymphoid- and plasma-cell series in the elaboration of excessive γ-globulin in cirrhosis suggests either a coordinated effort of two systems or, judging from the many intermediate forms, a transition between the two cell types.

The role played by the spleen in the production of excess γ-globulin is also suggested by the frequent association of splenomegaly and a markedly elevated serum γ-globulin in human postnecrotic [2] and experimental cirrhosis.[29] In this study no correlation between spleen weight on the one hand and either pyroninophilia or serum γ-globulin values on the other was evident. This is not surprising in view of

the many other factors which influence the size of the spleen.

This study throws no light on the contribution of either the liver or the bone marrow to the hypergammaglobulinemia because of the variety of pyronin-staining cells seen in these organs in the material studied. Further work particularly with the fluorescent antibody technique is required for the elucidation of this question. Such studies would perhaps also confirm the conclusion made here about the role of lymph nodes and spleen in the production of γ-globulin in cirrhosis. It also remains to be determined to what degree the observations made apply to the hypergammaglobulinemia of nonhepatic origin.

Summary

The distribution and morphology of the cells with cytoplasmic pyroninophilia removed by ribonuclease and therefore presumably active in protein synthesis was studied in spleen and lymph nodes of normals and of patients with diffuse septal and postnecrotic cirrhosis. The pyroninophilic cells in the splenic white pulp and in lymphoid follicles do not differ significantly in cirrhosis from those in normals. The splenic red pulp and medullary cords of

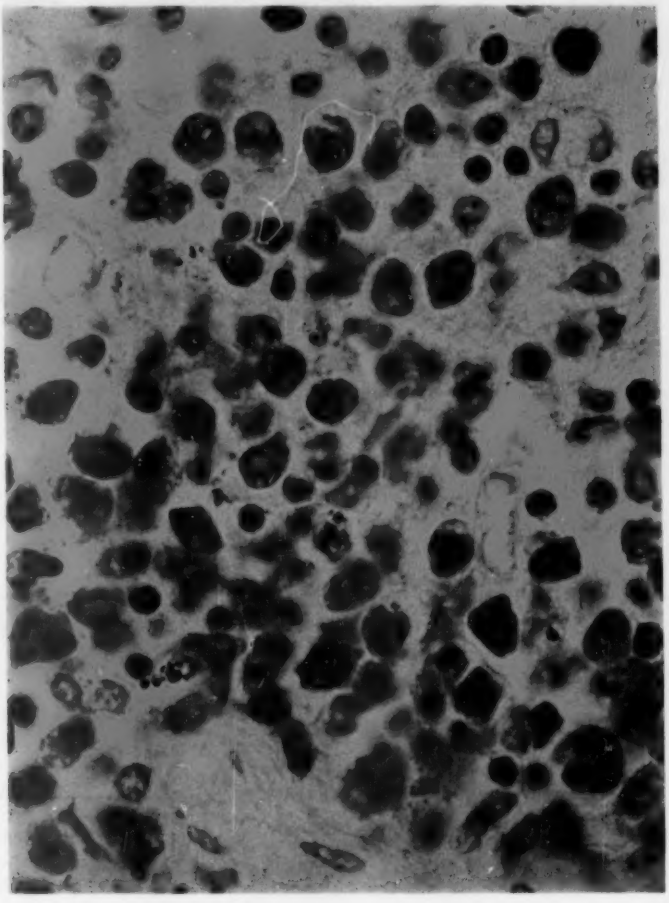

Fig. 6.—Lymph node in cirrhosis. Large cluster of pyroninophilic cells, showing various cell types. Methyl green and pyronin stain; × 1040.

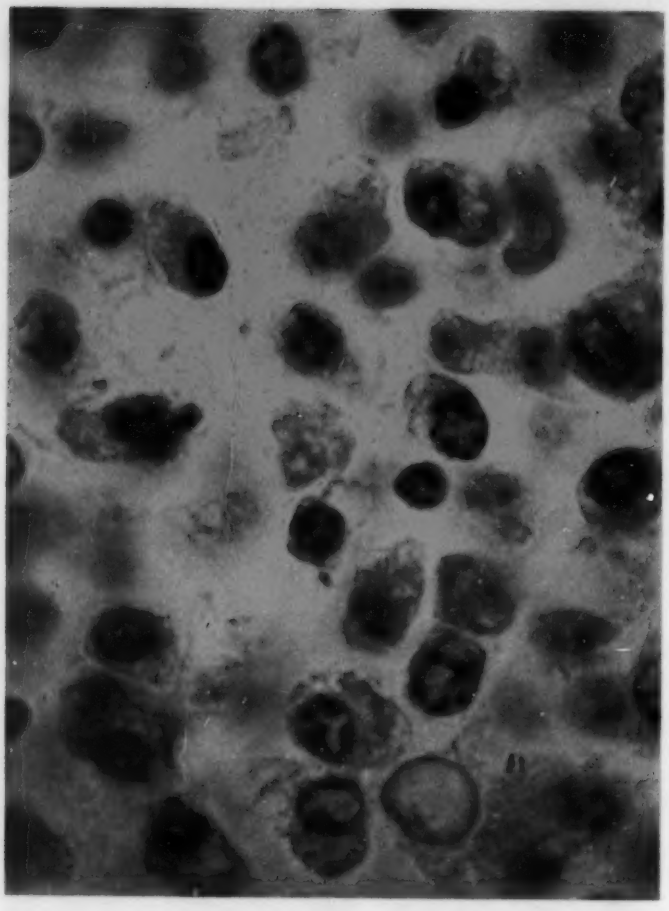

Fig. 7.—Lymph node in cirrhosis. Nuclear detail of pyroninophilic cells from cluster in medullary cord. Somewhat greater variation in cell type is found in the spleen. Methyl green and pyronin stain; × 2000.

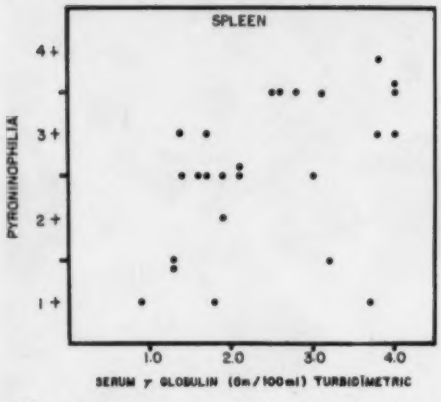

Fig. 8.—Relation of splenic pyroninophilia to serum γ-globulin levels.

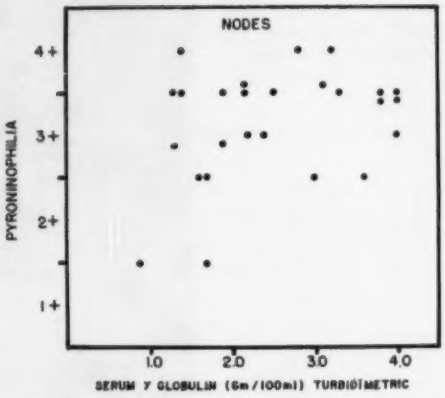

Fig. 9.—Relation of lymph node pyroninophilia to serum γ-globulin levels.

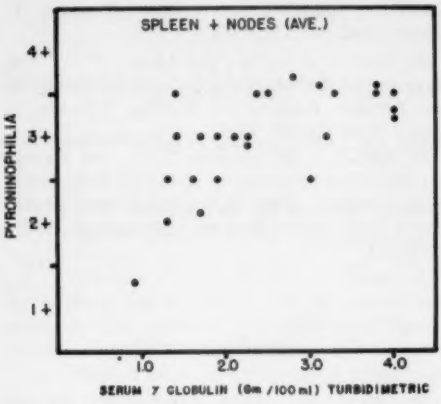

Fig. 10.—Relation of averaged spleen and lymph node pyroninophilia to serum γ-globulin levels.

lymph nodes, in contrast, reveal relatively few, usually isolated, pyroninophilic cells in normals and a marked increase of such cells, usually arranged in clusters, in cirrhosis. The pyroninophilic cells belong to both the lymphoid- and plasma-cell series and show many intermediate forms. The distinctive pattern of the pyroninophilia and its correlation with serum γ-globulin levels suggests that the spleen and lymph nodes represent sites of γ-globulin production in cirrhosis.

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Dissecting Hemorrhage in Media of Coronary Artery

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This report concerns an unusual lesion, dissecting aneurysm of the right coronary artery, which caused sudden death of a relatively young woman.

Clinical History

The patient was a 41-year-old white housewife who had always been in good health. On the morning of Sept. 20, 1954, she complained of pain in the "lower part of the chest" but continued with her usual household activities. By noon the pain became localized under the sternum and radiated down the left arm. Suddenly, while talking to her husband, she collapsed and became unconscious. She was taken to the hospital but was dead upon arrival. No other pertinent information, including taking of any drug, was obtained.

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Supported by a grant from the National Heart Institute to Wayne State University College of Medicine (Dr. S. E. Gould, principal investigator).

From the Departments of Pathology, Foote Memorial Hospital, Jackson, Mich.; Wayne County General Hospital, Eloise, Mich., and Wayne State University College of Medicine, Detroit.

Autopsy Findings

Gross

The heart was small, and the pericardial surfaces were smooth and glistening. A small area of congestion was present over the posterior descending branch of the right coronary artery. The coronary arteries were thin-walled, with no evidence of narrowing of their lumens. An area of hemorrhage was present in the wall of the proximal portion of the posterior descending branch of the right coronary artery; the lumen was narrowed but not occluded. The ventricles were of normal size, without evidence of hypertrophy, and the myocardium was red-brown throughout. The valves were delicate. The aorta, trachea, and esophagus were hypoplastic. Both lungs were emphysematous and appeared bright red in color. The other organs, including the brain, showed no remarkable gross findings.

Microscopic

Stepsections of the posterior descending branch of the right coronary artery show a large area of recent hemorrhage in the outermost layers of the media (Fig. 1). Sections stained by the Verhoeff and Van Gieson

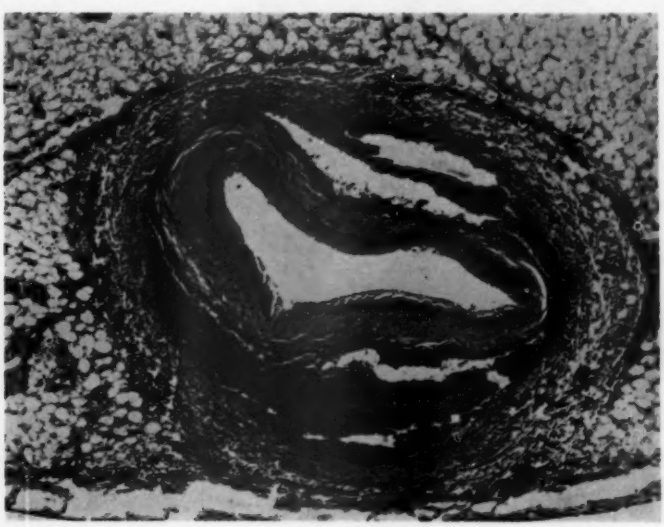

Fig. 1.—Section of coronary artery, showing hemorrhage in the outer portion of the media. The media is encircled by hemorrhage, except for an area in the upper left. Note cuff of inflammatory cells encircling and extending into adventitia. Hematoxylin and eosin; reduced 20% from mag. × 35.

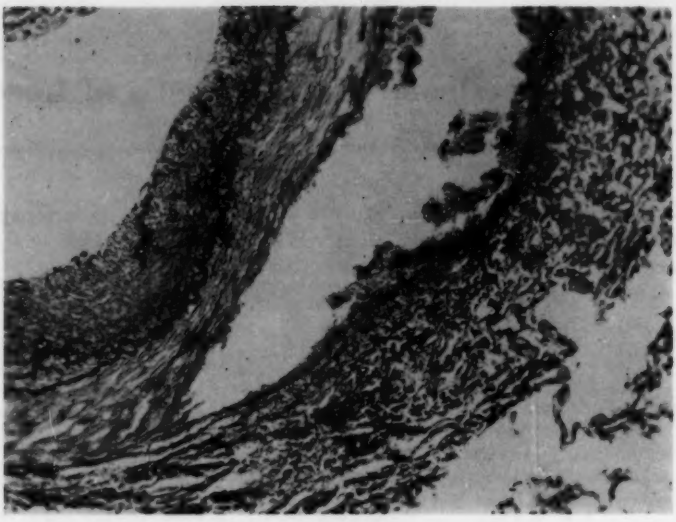

Fig. 2.—Portion of artery, showing dissection in outer portion of media. Verhoeff-Van Gieson stain; reduced 20% from mag. × 160.

method show the hemorrhage to be bordered externally by the external elastic lamina and in some areas by a layer of one or two cells of smooth muscle (Fig. 2). The hemorrhage is recent, consisting of well-preserved red blood cells without lysis. The lumen of the artery is reduced to approximately one-third of its estimated original cross sectional area. The area occupied by the hemorrhage is larger than that of the reduced lumen. The intima is slightly thickened by fibrous and hyaline connective tissue, but there is no atheroma or evidence of thrombosis. Encircling the adventitia is a cuff of heavy infiltration with eosinophils, plasma cells, lymphocytes, histiocytes, and occasional polymorphonuclear leukocytes (Fig. 3). This infiltration also involves the adventitia and

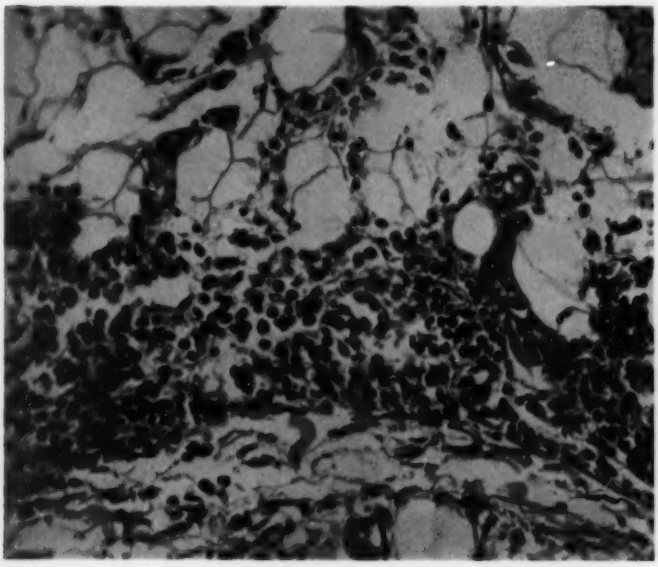

Fig. 3.—Portion of cuff of inflammatory cells bordering adventitia, consisting of eosinophils, plasma cells, lymphocytes, histiocytes, and occasional polymorphonuclear leukocytes. Hematoxylin and eosin; reduced about 15% from mag. × 340.

the adjacent subepicardial adipose tissue. The adventitia is hyperemic, and its fibrous connective tissue seems to be fragmented and of hyaline appearance. The intima and media show no infiltration with inflammatory cells. In the media are seen no areas of necrosis and no apparent increase of basophilic ground substance. Stains for bacteria, fungi, and spirochetes are negative. The myocardium throughout is not remarkable, except for slight to moderate periadventitial fibrosis of occasional vessels.

A section of lung shows congestion and recent focal hemorrhage. The other organs show no remarkable features.

Comment

The origin of the hemorrhage remains obscure, no connection with the lumen having been demonstrated. In arteries of this size the vasa vasorum penetrate the external layers of the media, and hemorrhage from rupture of one of these vessels cannot be excluded. Although the lesion resembles a dissecting aneurysm, this term would not be correct; the dissection did not originate from the lumen, and there was no widening of the artery, as the word "aneurysm" would imply.

The cause of death in this patient is not clearly established. Microscopically there was no evidence of thrombosis or recent infarction. The hemorrhage separates the outermost portion of the media from the remainder of the media for approximately five-sixths of the circumference of the vessel. The hemorrhage, however, extends into both sides of the portion of media that is still attached, leaving an area of less than one-tenth of the circumference free of hemorrhage. The lumen of the affected artery was compressed by the medial hemorrhage to one-third its estimated original area. The question may be raised whether spasm of the media during life had further reduced the lumen. In any event, most of the media, over most of the circumference of the vessel, was detached from its nutrient vasa vasorum coming from the adventitia. Evidence of anoxemic necrosis of the media, however, was not apparent. Either the hemorrhage in the wall or the reduction in blood flow, or both together, may have led to a so-called "mechanism death."

The character of the periadventitial infiltrate suggests a periarteritis nodosa or sensitivity type of angiitis. Periarteritis nodosa is the commonest cause of coronary artery aneurysm. Although periarteritis nodosa of coronary arteries is usually part of a generalized arteritis, its occurrence without involvement of other organs has been described in infants.[6] Periarteritis nodosa is generally characterized by medial necrosis and inflammatory infiltration of all layers of the vessel wall, but neither of these features was present in our case.

Aneurysms of the coronary arteries not associated with periarteritis nodosa are rare and are classified by Scott [5] as being chiefly congenital, mycotic-embolic, or arteriosclerotic. In these cases death is most commonly attributed to rupture or thrombosis of the affected artery or to associated myocardial disease. Only a few instances of dissecting aneurysm of a coronary artery have been reported. Except for one case, reported by Pretty,[4] these have resulted from extension of hemorrhage from a dissecting aortic aneurysm.[8] Pretty described an isolated dissecting aneurysm of the right coronary artery in a 42-year-old woman in whom death resulted from rupture and implied that the aneurysm was of atherosclerotic origin. Unfortunately, no microscopic description is given in his report.

In Marfan's syndrome, dissecting aneurysms of the aorta and other larger arteries have been reported [5]; in one case, a dissecting aortic aneurysm encircled the right coronary artery.[9] Aortic hypoplasia, which was present in our patient, has been described twice in Marfan's syndrome.[7,9] Since visceral lesions without obvious skeletal or ocular defects may be the only evidence of Marfan's syndrome,[9] this disease must be excluded in our case. In Marfan's syndrome, however, arteries much larger than

the coronary are involved; medionecrosis is prominent, and no characteristic inflammatory infiltrate is present.

The possibility of a traumatic or parasitic origin of this lesion is improbable but cannot be empirically excluded.

Kernohan and Woltman [2] described four cases of focal necrosis of a vertebral or cerebellar artery with rupture following uncomplicated abdominal operations. These vessels showed acute necrosis of the adventitia which in some cases extended into the media. The necrosis in the adventitia was associated with infiltration of polymorphonuclear leukocytes. The media and intima were usually uninvolved. One of their cases presented areas of dissecting hemorrhage between the outer portion of the media and the adventitia. In the local areas of involvement of affected arteries, the intima was intact and the source of the hemorrhage was not found. They believed this process originated in the adventitia and speculated that possibly occlusion of vasa vasorum was the initial lesion. Gruenwald [1] described medial necrosis of coronary arteries in newborn and stillborn infants and postulated that the lesion in his cases and those of Kernohan and Woltman may have been caused by anoxia.

The subacute to chronic nature of the infiltrate in our case indicates its occurrence prior to the recent hemorrhage. Thus, our case closely resembles those of Kernohan and Woltman in that the process apparently started in the adventitia and was limited to one artery and the origin of the dissecting hemorrhage could not be demonstrated. It differs from their cases in the location of the artery and in the heavier and more chronic nature of the infiltrate. In their cases the vessels ruptured early and resulted in fatal subarachnoid hemorrhage, while in our patient the underlying lesion may have been of longer duration, since the infiltrate was of the more chronic nature. In our case, as in theirs, few vasa vasorum were seen and none showed any significant change.

Despite the lack of information concerning a possible etiologic agent, it is thought that the basic lesion was periarteritis nodosa, apparently limited to the right coronary artery.

The material was submitted to Dr. Otto Saphir, of Chicago, whose opinion follows: The two available sections show an obvious dissecting aneurysm of the coronary artery. Parts of the media are destroyed. The blood vessel itself is surrounded by many inflammatory cells, principally monocytes, eosinophilic cells and also polymorphonuclear leukocytes. As far as the intact portion of the media is concerned, there seems to be some fluid material (edema?) separating individual fibers. There is a slight thickening of the intima and an inconspicuous infiltration of lymphocytes, most likely part of a mural thrombus.

Grossly, there apparently was no tear in the intima anywhere. It would have been helpful to study other coronary arteries and sections of the aorta for evidence of medial degeneration or necrosis. It has been stressed in the literature that where there are dissecting aneurysms in the absence of intimal tears; these may be explained by rupture of the vasa vasorum. I believe that the latter occur only in so-called Erdheim's medial necrosis. The inflammatory cells surrounding the blood vessel are obviously older than the tear in the media itself. The many eosinophils that are present may speak for a hyperergic element.

Summary

A dissecting hemorrhage in the media of the ramus interventricularis posterior of the right coronary artery caused sudden death in a 41-year-old woman. The nature of the periadventitial inflammatory infiltrate suggests periarteritis nodosa, but this lesion was not found in any other artery examined at autopsy.

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A Case of Bilateral Multicentric Cardiac Myxoma

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The clinical course and morphological details of the rare entity of cardiac myxoma have been well documented and reviewed most recently by Mahaim [1] and Prichard.[2] Of approximately 350 primary cardiac tumors reviewed by Prichard, in 1951, from the world literature, 126 were myxomas and the remainder were sarcomas. Of course all myxomas have not been documented, and the cases of myxoma reported since 1951 have been justified mainly because of diagnosis prior to death and surgical attempts with varying degrees of success. The nature and histogenesis of this tumor are unsettled, as will be attested by the variety of names and theories, which will be discussed later. The purpose of this case report is not to add to the clinical picture, the morphological detail, or review the literature but instead to record a case with bilateral and multicentric foci and to attempt to clarify the histogenesis which at present is controversial.

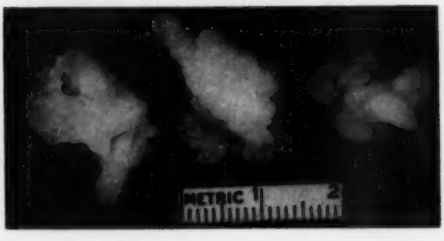
Case History

This is the case of a 19-year-old woman who 24 months prior to death was said to have had a sore throat, with pain on swallowing. Two months later she was said to have had pain, redness, and swelling in the right wrist and pain in the legs and lumbosacral region. Therapy consisted of bed rest and acetylsalicylic acid. Six months later the patient suffered a permanent right hemiplegia with aphasia and considerable deterioration of the intellect. The patient continued to be bedridden and presented a symptom-complex thought to be characteristic of rheumatic heart disease and which rapidly progressed. During this time she was treated with bed rest, salicylates, prednisone (Meticortin), digitalis, acetazolamide (Diamox), and symptomatic therapy, in addition to physical therapy for the hemiplegia. The patient during the last 10 months was hospitalized and complained frequently of pains in the legs and chest; associated with the latter were periods of dyspnea and apnea, during which oxygen was administered. X-rays and electrocardiograms taken during the last months were interpreted as showing right heart hypertrophy. Tachycardia was present, and during the last months auricular fibrillation was intermittently present. Systolic and diastolic murmurs of varying character and intensity were heard which frequently changed in character as the patient assumed different positions. On the day previous to death, following an attempted left atrial tap, the patient developed arterial occlusion to both lower extremities. Embolectomy yielded an irregular gelatinous "clot" 4 cm. in length and 1-1.5 cm. in diameter (Fig. 1). The patient did not recover consciousness and died in respiratory distress.

Autopsy Findings

The heart weighed 325 gm. The most conspicuous feature was a soft yellowish cauliflower-like fungating mass composed of fronds and filling most of the left atrium (Fig. 2). This structure was attached to the superior margin of the limbus of the foramen ovale; parts protruded thru the mitral valve. This mass was approximately spherical, measuring 6-8 cm. in diameter. It was attached to the superior aspect of the distorted limbus by a fibrous pedicle 1 cm. in diameter. Attached to the

Fig. 1.—Myxoma embolus removed from bifurcation of aorta one day prior to death. The single piece was cut into three in order to obtain tissue for surgical diagnosis. Photographed by courtesy of Dr. Saul Kay.



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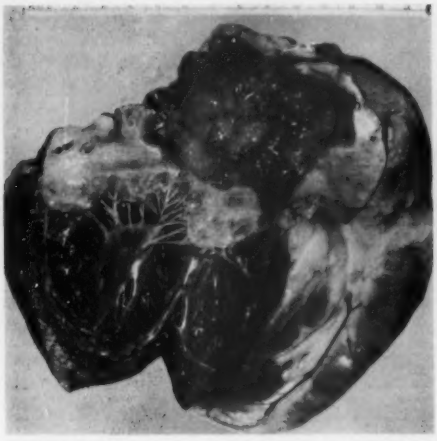


Fig. 2.—Main mass of myxoma filling left atrium.

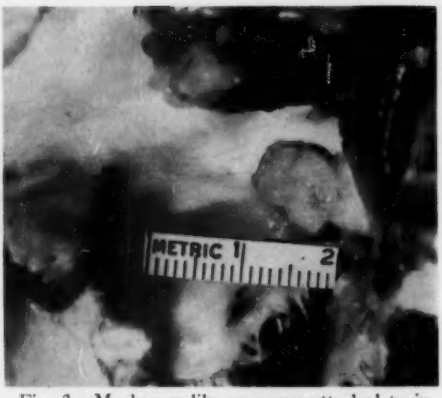


Fig. 3.—Mushroom-like myxoma attached to inferior aspect of limbus of foramen ovale on left. The dark mass above is part of the hemorrhagic main tumor resected to obtain a view of the limbus.

limbus in its inferior and anterior aspects on the left side were four small cylindrical polyp-like myxoma masses, each measuring 5 mm. in length and 1-2 mm. in diameter. Also present was a single flattened mushroom-like tumor measuring 8 mm. in diameter and 2 mm. in thickness, attached by a stalk 2 mm. in diameter and 2 mm. in length to the limbus of the foramen ovale (Fig. 3). The surface of the fronds was smooth and glistening and showed no gross evidence of thrombus. When the myxoma was sectioned, some focal areas of hemorrhage were seen. The auricular appendages were free of thrombi. The mitral valve ring after fixation in Kaiserling's solution measured 11 cm. in circumference. The valve cusps were normal, being thin and delicate; the papillary muscles were, perhaps, slightly thickened and shortened. The left ventricle measured 14 mm. in thickness at the base of the anterior papillary muscle. The left ventricle and aortic valves were normal; the aortic ring measured 6 cm. in circumference. In the right atrium at the posterior aspect of the limbus of the imperfectly fused foramen ovale there was a single bud-like hemorrhagic myxoma protruding into the chamber. This tumor measured 3 mm. in diameter and 9 mm. in length

Fig. 4.—Arrow points to bud-like hemorrhagic myxoma growing from cleft of imperfectly fused foramen ovale on the right.

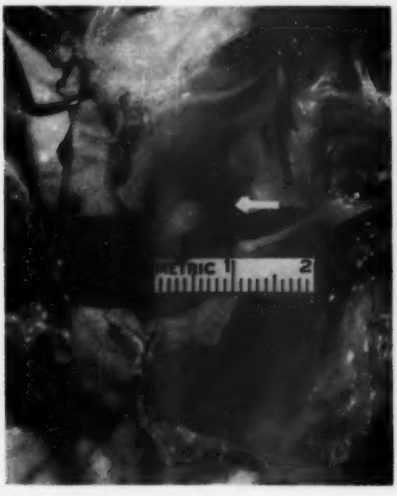
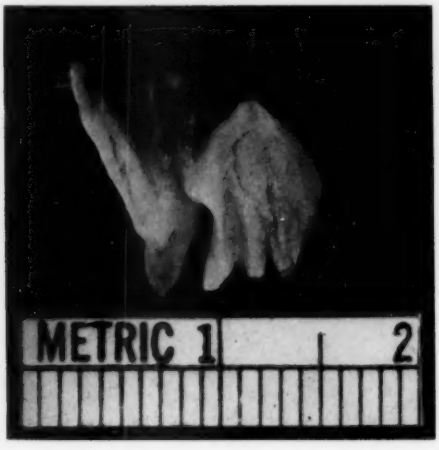


Fig. 5.—Section of bud-like myxoma from Figure 4, showing in detail the manner of growth from the imperfect fusion of the foramen ovale.



(Figs. 4 and 5). The remainder of the atrium and auricle was normal and without thrombi. The right ventricle measured 6 mm. in thickness and was grossly normal. The pulmonic valve was normal and measured 8 cm. in circumference. The pulmonary artery was thickened and showed yellow plaques of atherosclerosis.

Gross inspection of other organs revealed old and recent infarcts of the kidneys, spleen, and lungs. Recanalized thrombi were present in medium-sized pulmonary arteries. The brain showed cystic spaces in the caudate nucleus, claustrum, globus pallidus, putamen, and internal capsule on the left; these measured 1-2 cm. in diameter. Recent hemorrhage and softened areas 0.5-1 cm. in diameter were distributed randomly throughout the remainder of the brain. Thrombi were not found in the larger intracranial arteries. However, the right internal carotid artery did not transmit water, while the left transmitted 550 cc. per minute with a 30 cm. head of pressure. Both vertebrals transmitted 380 cc. per minute with the same head of pressure. Dissection of the right carotid artery above the level of the hyoid bone and below the level of the siphon was not obtained; thrombi were not present in the portions obtained.

Histological Features

The histological features of this myxoma are in good agreement with similar such cases, as reviewed by Prichard. The main tumor mass was composed of uniform myxoid tissue characterized by a loose edematous mesenchyme-like stroma which stained poorly with hematoxylin and eosin and portions of which gave a positive reaction for mucin. The cells in this stroma were, for the main part, stellate, with poorly defined cell boundaries resembling those of Wharton's jelly and fibroblasts growing in tissue culture (Fig. 6). A few poorly formed single and multinucleated giant cells

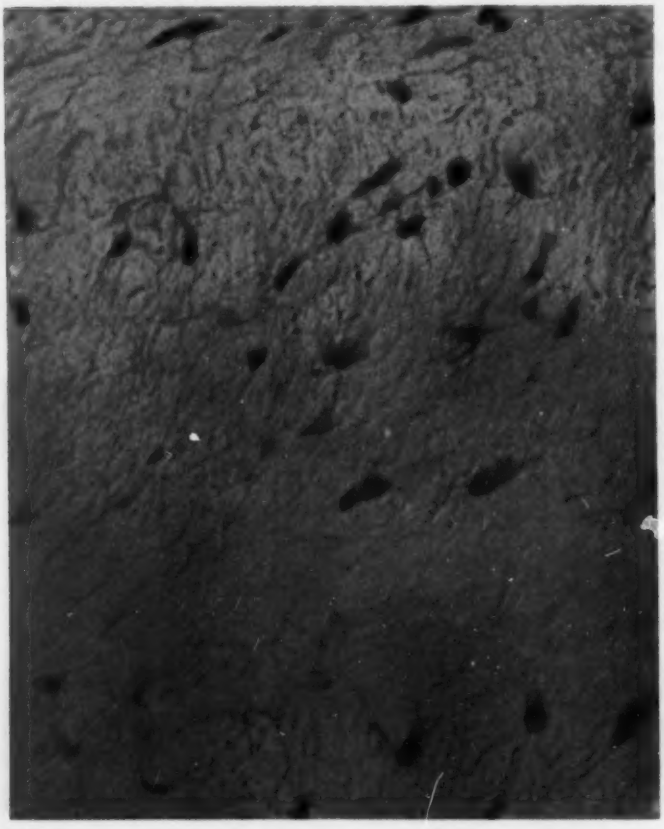


Fig. 6.—Microscopic section of representative area from myxoma, showing stellate cells in a loose stroma appearing not unlike that of Wharton's jelly. Hematoxylin and eosin; × 500.

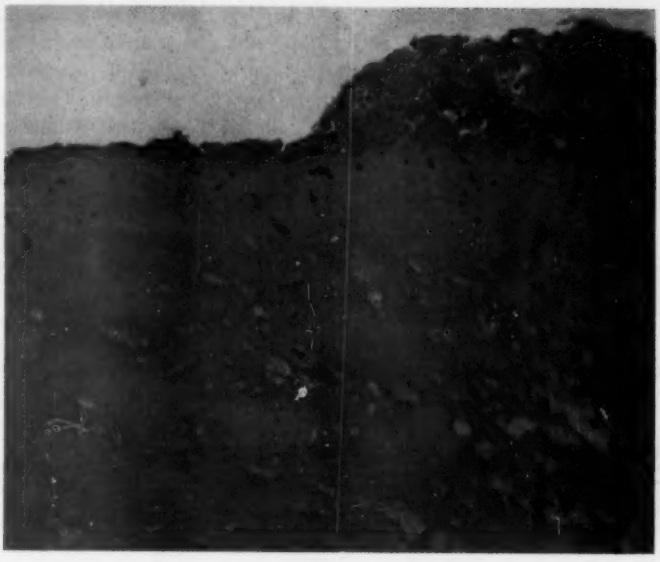


Fig. 7.—Microscopic section of another area from myxoma, showing smudgy ischemic appearance in the interior and better preserved cells immediately beneath the single cell layered surface. Also note a fibrin thrombus on the surface. Hematoxylin and eosin; × 120.

were present in the stalk, where fibrous tissue with bands of collagen were present and which extended into the attachment to the myocardium. Focal collections of lymphocytes were seen, and a few areas showed calcification. Elastic stains revealed a few elastic fibers distributed in a random fashion.

In the center of the fronds away from the vascular base there were areas which appeared to be undergoing ischemic necrosis in that the ground substance had a smudgy appearance and the few cells present were undergoing cytolysis; these areas were devoid of capillaries. The outermost 2 mm. of the tumor beneath the surface had the best-preserved cells. The surface of the tumor was covered with a single layer of flattened endothelial cells and was free of thrombi except for a very few areas (Fig. 7). The attachment of the tumor by its fibrous stalk was characterized by a delicate blending of the fibrous strands of the stalk with those of the sheaths of the myocardial bundles and the fibrous tissue of the perivascular spaces. In the case where the two flaps of the foramen ovale failed of perfect union the base of the tumor extending into the right atrium was identical with the fibrous tissue composing this union. This tumor was characterized by the extensive recent hemorrhage. No evidence of inflammation was found in any of the sections of the tumor studied, and hemosiderin was absent.

Sections from many portions of the myocardium showed extensive fibrosis especially in the perivascular clefts, in which many foci of lymphocytes were present. However, typical Aschoff nodules with Anitschkow cells were not found. Hypertrophy of the muscle fibers was prominent, especially in sections from the right atrium. Sections of both auricles failed to reveal any evidence of thrombi.

Sections from the embolus removed prior to death showed it to be composed of tumor tissue identical to the primary tumor. Sections of the spleen showed tumor tissue emboli in several of the larger vessels adjacent to the infarcts. The sections of the kidney showed extensive infarcts and showed many of the arcuate and interlobar arteries to be filled with tumor emboli. The cellular pattern of these emboli was well preserved and was identical with that of the primary tumor. The emboli in the lung appeared to be ordinary recanalized clots, presumably from the hemiplegic extremity.

Comment

From the various terms, such as myxoma, myxofibroma, elastomyxoma, fibroangiomysoma, hemangioelastomyxoma, fibroelastomyxoma, and fibromyxosarcoma, all of which have been applied to this tumor, it is clear that the nature of the lesion is poorly understood. This is partly due to the rare occurrence of the tumor and the fact that no one investigator can acquire more than a few specimens for comparison. The literature consists mainly of single case reports, and the histological description in most of the early cases is too meager and confusing to permit the drawing of definite conclusions.

The origin has been considered uncertain; the early writers considered these tumors to be a type of "peculiarly organizing" thrombus. This was put forth mainly by Thorel, in a series of papers which were based on the previously published literature. The last of these papers appeared in 1915.[3] The nature of the tumor and ordinary thrombi of the heart was not clearly delineated or suspected at that time, and because of the rarity of the tumor there was no need to establish a new histological entity. From the histological standpoint and on first consideration some support for this view can be had. There are in some areas of many of these tumors hemorrhage and hemosiderin together with conspicuous vascular proliferation. The hemorrhage is no doubt partly due to the fact that these tumors are soft and friable, as evidenced by frequent emboli, and are constantly subjected to mechanical trauma of the blood stream while hanging from a peduncle attached to a rapidly expanding and contracting heart. The fact that these tumors are soft and without capsule or other confinement probably accounts for the fact that the mesenchymal cells grow in a stellate pattern as in tissue culture. However, it must be borne in mind that the usual thrombus forms in the auricles, and no myxomas have been reported in the auricles. The fact that the majority of myxomas occur in the left atrium and are attached to the limbus of the foramen ovale bespeaks a different etiology, as does the fact that myxomas have not been correlated with any of the usual causes of thrombi, such as myocardial infarcts, rheumatic disease, and fibrillation.

The Table, adapted from Mahaim, shows the comparison of myxomas with thrombi.

A further consideration of the literature reveals that these myxomas may behave in a manner dissimilar to the thrombus, thus Ringertz[4] reports a case in which the embolus from a myxoma continued to survive and became encapsulated. Haythorn et al.[5] describe a case of "fibromyxosarcoma" which arose at the base of the pulmonary artery and metastasized.

In view of the fact that most myxomas arise at the limbus of the foramen ovale, it is logical to seek a cause here. It is known that fibrous tissue in the heart is present to the greatest extent at the site of the former foramen ovale. Kesserling,[6] cited by Thorel,[7] early suggested that these tumors arise from embryonic myxoid rests,

Comparison of Myxomas with Thrombi

Myxoma	Organizing Thrombus
Surface smooth, glistening, & transparent	Surface granular & opaque
Consistency soft & gelatinous	Substance friable & stratified
Stroma uniform, amorphous, colorless, & with but few cells, which are distributed randomly & not in a synctium or groups	Stroma composed of network of proliferating & organizing capillaries
Absence of hemosiderin in most of areas of most tumors	Hemosiderin & fibrin abundant
Mucin positive	Mucin negative
Elastic fibers present around vessels of tumor; pedunculated	Endocardial lesion at base of thrombus, wide base
Substance continuous & blends with myocardium, covered with endocardium	Demarcated from myocardium & not covered with endocardium
Located above left atrioventricular valves, especially at limbus of foramen ovale	Located in both auricles

and Ribbert[8] after making an extensive study of the histological features of this region, concurred in this view. Prichard, in a study of 55 foramen, did not find the rests reported by Ribbert but did in 4 of his cases (8%) find endothelial deformities of a "hemangiomatus" nature, not unlike some areas of the vascular components of the myxoma of this case. That the endothelial surface of the heart can develop myxomatous tissue is suggested by Warthin,[9] who reports such nodules on the endocardial surface of the heart in cases of congenital syphilis.

The finding in this case of a multicentric tumor with all of the pedicles attached to the limbus of the foramen ovale on the left side of the heart and a similar tumor growing from the cleft of the imperfect closure on the right speaks strongly for the neoplastic nature of the tumor and against the "organizing thrombus" view. It is very difficult to conceive of this multicentric and bilateral tumor with its fibrous and vascular pedicle continuous with the fibrous tissue of the limbus as having an origin from a thrombus, especially since the heart showed no evidence of thrombi or any underlying cause for a thrombus. The patient had an embolus to the brain before the rheumatic heart disease was diagnosed and 16 months before fibrillation was present. This would seem to indicate that the tumor was present prior to the rheumatic episode; in retrospect the whole illness can be explained on the basis of the myxoma without implicating rheumatic disease, the diagnosis of which was not overwhelmingly supported at autopsy apart from the interstitial myocarditis.

Summary

The case of a 19-year-old girl is described in which 18 months prior to death there had been an embolus to the brain, at which time no evidence of carditis was present. At autopsy multicentric bilateral cardiac myxomas were found in which all of the pedicles of all the tumors were continuous with the fibrous tissue of the limbus of the foramen ovale. No evidence of thrombi was found. The multicentric and bilateral aspects of the tumor are interpreted to indicate that cardiac myxomas are true neoplasms, arising without relation to prior thrombi.

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Recovery of the Rat Kidney in Fluorosis

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Introduction

In a previous paper[4] it was shown that a diet containing 0.05% sodium fluoride caused histopathologic alterations in the kidneys of rats within 21-28 days. These changes consisted primarily of dilation of Henle loops and were followed by a flattening of the epithelium in the convoluted tubules. Long-term experiments with high dosages of fluoride demonstrated that these changes developed into inflammation, interstitial fibrosis, and marked destruction of the tubules.[1-3,5]

Since we were unable to find reference to any investigation showing whether the kidney changes in fluorosis were reversible, experiments were designed to evaluate this factor.

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From the Biological Laboratories of Medicinalco, Ltd., and the Department of Histology, the Royal Dental College.

Material and Methods

(a) Tissue Recovery in Six Months.—Forty-eight 2-2½-month-old white rats of the Wistar strain were divided into three equal groups. All animals received the same stock diet, composed of dry skim-milk powder, 35%; rye meal, 35%; wheat bran, 11%; dry yeast, 8%; arachis oil, 11%; shark-liver oil, 3000 I. U. vitamin A per kilogram. Group I received 0.05% sodium fluoride added to the stock diet for 61 days and were then fed the stock diet without fluoride for periods varying between 14 and 175 days. The rats in Group II received 0.05% sodium fluoride continuously and were killed simultaneously with the rats in the first group. In Group III 16 rats acted as controls, receiving only the stock diet.

(b) Tissue Recovery in One Year.—In this experiment twenty-eight 2-month-old rats in Group IV received 0.05% sodium fluoride in the diet for 58 days and were then fed the stock diet for periods varying between 91 and 384 days. Six rats (three male and three female) got the stock diet throughout 58+384 days (Group V). Among the experimental rats three died before the end of the experiment and four were so ill that they were killed.

In all the groups the rats were individually housed and weighed twice a week. The diet and

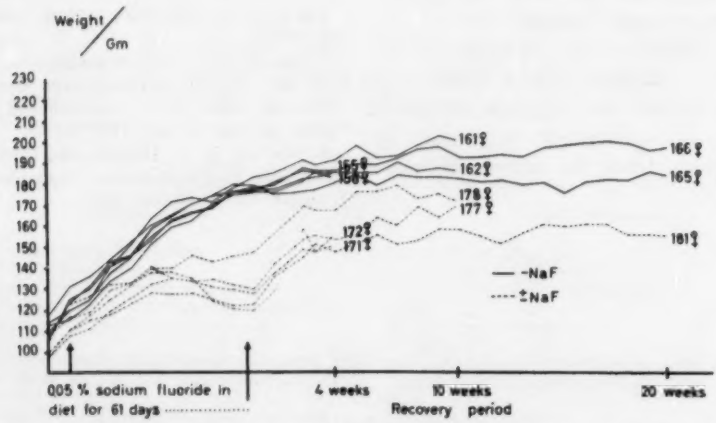

Fig. 1.—Weight curves for female rats in Group I and in Group III. The distance between the arrows indicates the period during which the rats in Group I received sodium fluoride.

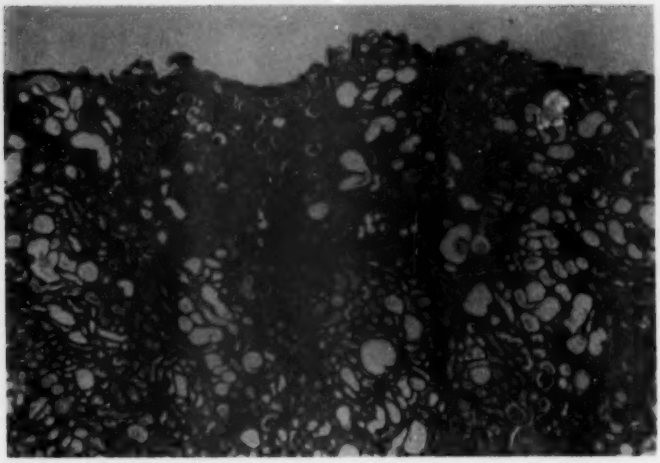

Fig. 2.—Low-power magnification of kidney from a rat which has received fluoride for 173 days. Note the irregular surface, the fibrotic areas, and the dilated tubules.

tap water were available ad libitum. The amount of food consumed was computed every day and the average amount of fluoride ingested was calculated. The kidneys were removed at autopsy, fixed in 4% formalin, embedded in paraffin, cut, and stained with hematoxylin and eosin and Van Gieson's connective tissue stain.

Results

Weight.—The weights of the animals in Group I compared to those of the control group (III) showed that the animals in Group I increased their weights considerably in the first two or three weeks after withdrawal of fluoride from the stock diet. After this period, their weights increased very slowly but did not reach the weights of the controls. This is illustrated in Figure 1 for the female rats in Group I and the corresponding female rats in Group III. In Group IV no significant increase in the weight after withdrawal of fluoride, as compared with the controls in Group V, could be found.

Histopathology of the Kidneys.—All animals in Group II, which received the fluoride throughout the entire experimental period, revealed kidney changes histologically typical of chronic fluoride intoxication. Macroscopically the surface of the kidneys was hobnailed and the kidneys were often

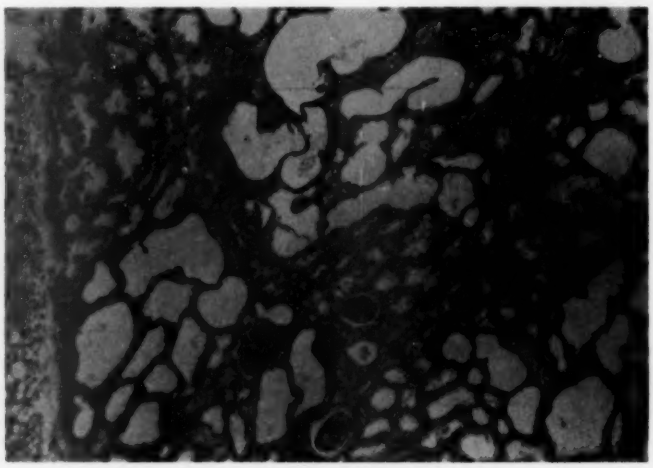

Fig. 3.—Higher magnification of area from Figure 2. Note the dilated tubules, the fibrosis, and the inflammation.

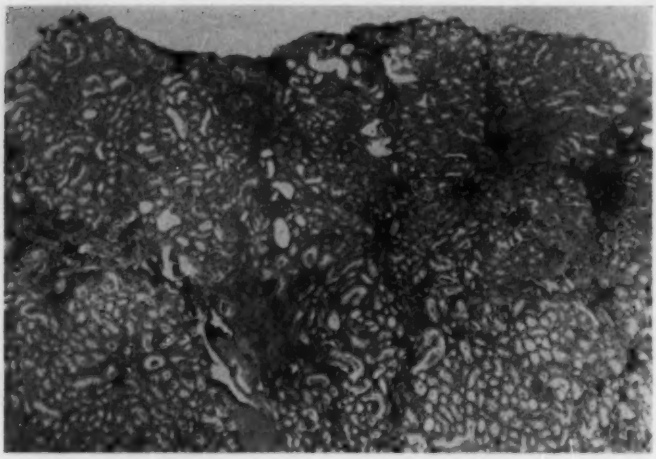

Fig. 4.—Low-power magnification of kidney from a rat which has received fluoride for 61 days and thereupon stock diet for 122 days. Note the diminished number of dilated tubules compared to Figure 2.

reduced in size. Microscopically, there was an extensive dilation of the Henle loops and convoluted tubules. Focal fibrosis was responsible for the hobnailed appearance (Fig. 2). A focal accumulation of lymphocytes and leucocytes was frequently observed (Fig. 3). In some areas there seemed to be some normal kidney tissue in both cortex and marrow. The longer the rat had been on the experimental diet, the more pronounced were the renal changes. The glomeruli were without demonstrable changes.

In Group I, where the rats received fluoride for 61 days and thereafter stock diet for varying periods, the animals revealed kidney changes corresponding to those in Group II until 70 days on the stock diet. However, after 112 days or longer, an improvement of the histopathologic changes of the kidneys could be observed in most of the animals. The inflammation was reduced in intensity, the dilations of the tubules were much less pronounced (Fig. 4), and the number of undamaged convoluted tubules was increased (Fig. 5). On the other hand, it should be noted that the fibrosis was unchanged in all animals.

The rats in the control group (III) did not show any kidney changes. In Group

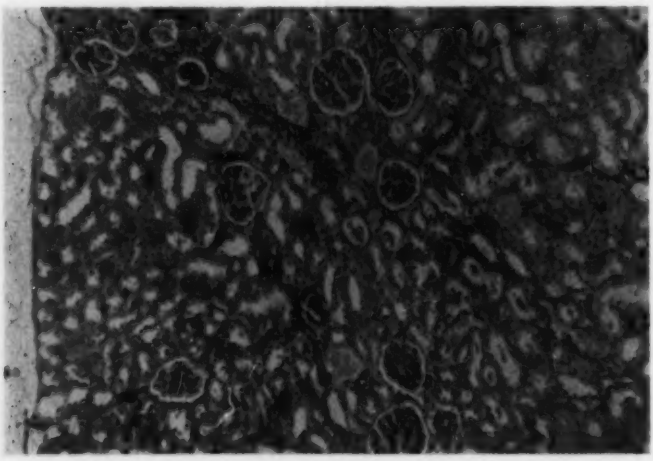

Fig. 5.—Higher magnification of area from Figure 4. Note the increased amount of undamaged tubules.

IV the kidneys from the three rats which died during the experiment and from the four rats killed before the end of the planned experimental period had severe histological changes identical to those found in Group II. Similar changes were found in the kidneys of the two rats killed just after the 58 days on the fluoride diet. Of the two rats kept on the fluoride-free diet for 91 days, one showed slight histological changes and the other, moderate changes. Both rats with the 180-day recovery period had histologically normal kidneys. Among the 15 rats kept 384 days on the fluoride-free diet after 58 days on a diet containing 0.05% sodium fluoride, 1 had severe histological changes; 4, moderate changes; 2, slight changes; 4, questionable changes, and 4 did not show any changes in their kidneys.

None of the six control rats in Group V showed any histological changes in the kidneys.

Comment

In a previous paper[4] it was suggested that the essential pathological mechanism of the "fluorosed kidney" is some kind of a blockage in the Henle loops with consequent dilation of the convoluted tubules. In the present paper it was not possible to trace the suggested mechanism further because the changes were too far developed. However, it might be said, the blockage in the Henle loops was related to a constant intake of fluoride, since the dilation of the tubules was reduced when the fluoride was withdrawn. It also comes within the realm of possibility that this assumed blockage could have resulted from the deposit of heavy soluble salts.

The sequence of the changes in the "fluorosed kidney" is dilation of the Henle loops, followed by dilation of the convoluted tubules and later by inflammation. During the recovery process the dilation disappeared first, followed by a slower reduction of inflammation. As would be expected, the amount of fibrosis was unchanged. Finally, it should be mentioned that a year after the cessation of excessive fluoride diet a minority of rats still had dilated Henle loops and convoluted tubules. In these cases the interstitial inflammation and fibrosis were most pronounced. It remains for future research to establish how much fluoride it is possible to give rats without creating irreversible kidney changes.

Summary

In experiments with 76 rats it was demonstrated that withdrawal of fluoride from fluoride-intoxicated rats caused an improvement in the interstitial inflammation and a marked reduction of the dilated convoluted tubules and Henle loops of the kidney.

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Experimental Aberrant Lipogenesis

IV. Production of Nonsudanophilic Fat

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The synthesis of sudanophilic lipid in tissue cells has been found to depend on the incorporation of an oleate substrate into the fat.[1-3] It is entirely possible, however, that tissue cells might synthesize from other fatty acids lipids which, under ordinary means of staining, are not sudanophilic. It seemed obviously desirable, therefore, to repeat with palmitates and stearates the experiments which had been done with oleates but to use a criterion other than that of ordinary sudanophilia for the identification of the product.

Procedure

With use of a procedure similar to that of the previous experiments, sodium palmitate and sodium stearate were (1) injected into the corneas of living rabbits, (2) injected into excised corneal buttons which were then incubated, and (3) added to the serum in which corneal buttons and liver buttons were incubated. The period of observation was 1-21 days in the case of the living rabbits and 1-3 days in the case of the incubation experiments. The amount of test substance injected into the living animal or into the excised corneal button was approximately 0.05 ml. of 1% sodium palmitate or stearate, while that added to the incubation medium was approximately 0.1 ml. of the same solution per milliliter of serum. The excised button weighed approximately 10 mg., and each button was incubated in 3 ml. of serum. The incubation temperature was 37 C. Control observations were made on pieces of tissue incubated in serum without added fatty acid and incubated in the standard serum-oleate medium which had previously been used.

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After a predetermined period the tissues were fixed in formalin (occasionally in osmic acid) and, for the most part, sectioned in the frozen state. The sections were variously treated as follows: stained with Sudan IV at room temperature and at 65 C, stained with various other dyes, and examined with polarized light before and after immersion in various solvents. Autoradiography with palmitate-1-C^{14} was also done in some of the experiments, as had previously been done with radioactive oleate. To study fluorescence, fresh tissue and tissue fixed in formalin was sectioned in the frozen state and examined with a high-pressure mercury-vapor lamp.

Comparative observations were made on the staining and solubility properties of tristearin and tripalmitin embedded in gelatin, fixed in formalin, and sectioned in a manner similar to that of the tissue buttons.

Results

Cornea.—The results are summarized in the accompanying Table.

Sudan: As had been previously observed, no significant sudanophilia occurred in corneal tissue which had been injected with palmitate and stearate in vivo or incubated in media fortified by palmitate or stearate (Fig. 1). Nor did staining at 65 C result in any Sudan uptake. Staining at higher temperatures was impossible because of disintegration of the tissue. Abundant sudanophilic globules occurred, however, in the epithelium and stromal cells when incubated in media containing added oleate.

Hematoxylin: The cytoplasm of cells incubated in stearate or palmitate solution were, in comparison with those incubated in oleate solution, faintly granular and hematoxylinophilic. These granules were, however, Feulgen-negative.

Cresyl violet: The cells which had been exposed to stearate and palmitate gave only

*Staining and Solubility Characteristics of Intracellular Lipids Produced by Incubation of Cornea in Various Fatty Acids**

	Stain						Unstained			Solubility †						
	Sudan IV	Cresyl Violet	Osmic Acid	Nile Blue (Rose Color)	P. A. S.	Hema-toxylin	Baker's	Birefring-ence	Fluores-cence	$CHCl_3$	Ether	100% Alcohol	Acetone	0.1 N NaOH	0.1 N KOH	0.1 N HCl
Oleate	++++	++	+++	++++	+++	0	+	0	++	V. S.	S.	S.	Sl. S.	I. S.	I. S.	I. S.
Stearate	±	±	0	±	+++	±	++++	++++	++++	V. S.	S.	Sl. S.	Sl. S.	I. S.	I. S.	I. S.
Palmitate	0	±	0	±	+++	±	++++	++++	++++	V. S.	S.	Sl. S.	Sl. S.	I. S.	I. S.	I. S.
Triolein	++++	0	+++	++++	0	0	0	0	+‡	V. S.	V. S.	V. S.	V. S.		I. S.	
(in gelatin)																
Tristearin	0	0	0	0	0	0	0	++++	++‡	V. S.	V. S.	Sl. S.	S.		I. S.	
(in gelatin)																

* The results are compared with the staining and solubility characteristics of triolein and tristearin, respectively.
† V. S. indicates very soluble; S., soluble; Sl. S., slightly soluble; I. S., insoluble.
‡ Enhanced by formalin fixation.

a weakly positive metachromasia, while those exposed to oleate gave a strongly positive metachromasia.

Osmic Acid: This was strongly positive in the case of oleate-induced lipids but absent in that resulting from palmitates and stearates (Fig. 2).

Periodic Acid Schiff: This procedure resulted in a positive take by the cells which had been exposed to the fatty acids but was approximately equivalent for palmitate, stearate, and oleate. It was essentially negative for cells incubated in serum only and in all cells after alcohol extraction.

Baker's Acid Hematein: Sections from tissue incubated in serum stearate or serum palmitate and then subjected to the chromate-hematein procedure of Baker showed a strong take by the epithelial and stromal cells. Sections from tissue incubated in serum oleate showed only a mild chromophilia.

Birefringence: Tissue which had been incubated in sodium stearate or palmitate regularly showed an abundance of fine crystals in the cytoplasm of the epithelium, stromal cells, and endothelium (Fig. 3). They were uniformly absent from tissues which were incubated in oleate or from tissue incubated in serum only. They nevertheless had the same distribution and proportionate abundance as did the sudanophilic globules in the oleate experiments. In the injection experiments they were to be found only in the cells adjacent to the area of injection, again analogous to the distribution of the sudanophilic globules in the oleate experiment.

It was thus evident from these experiments that palmitate and stearate resulted in the formation of a crystalline substance in the tissue cells analogous to the formation of sudanophilic globules in cells exposed to oleates. Moreover, autoradiographs with radioactive palmitate showed that the palmitate anion was incorporated in these cells which showed the birefringent crystals.

Fluorescence: Tissue which had been incubated in serum palmitate and serum stearate and then fixed in formalin showed a strong fluorescence, corresponding to the

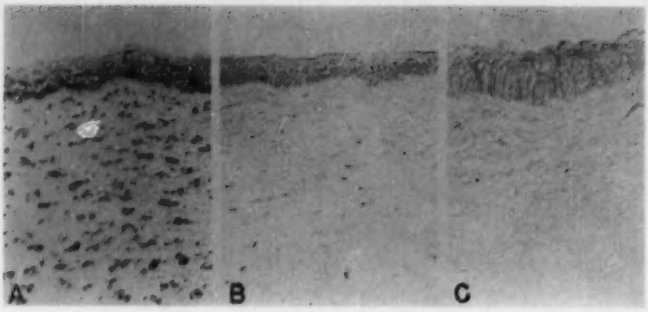

Fig. 1. — Sudanophilia of corneal sections after incubation for 24 hours in serum and *A*, oleate; *B*, stearate; and *C*, palmitate. The tissue incubated in serum oleate shows abundant sudanophilic material in the epithelium and stromal cells, whereas that which was incubated in serum stearate shows only a trace in the epithelium and that in the serum palmitate shows none. Sudan IV without counterstain; photographed with a 10× ocular and 16× objective. (The slightly greater thickness of the epithelium in *C* is an artifact due to oblique sectioning.)

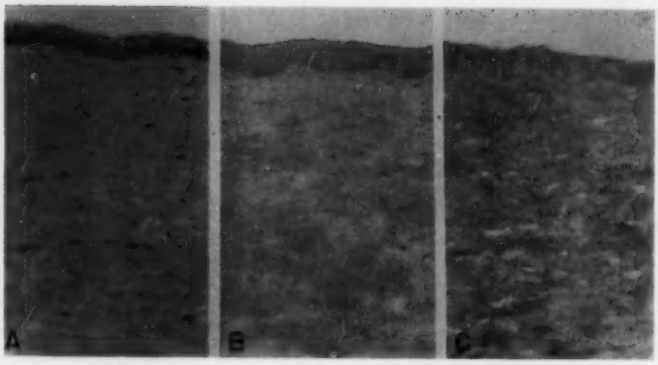

Fig. 2. — Osmic acid stain of corneal sections after incubation for 24 hours in serum and *A*, oleate; *B*, stearate; and *C*, palmitate. The tissue incubated in serum oleate shows abundant stained material in the epithelial and stromal cells, whereas that incubated in serum stearate and serum palmitate shows none.

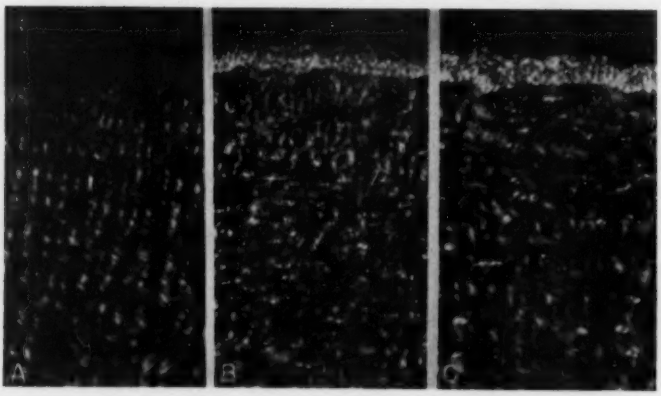

Fig. 3.—Birefringence in corneal sections after incubation for 24 hours in serum and *A*, oleate; *B*, stearate; and *C*, palmitate. Corneal stroma shows a herringbone type of birefringence normally, but in addition to this the tissue incubated in serum stearate or serum palmitate shows abundant birefringent crystals in the epithelial and stromal cells, whereas that incubated in serum oleate shows none. The photographs were made of unstained sections placed between crossed polaroid filters.

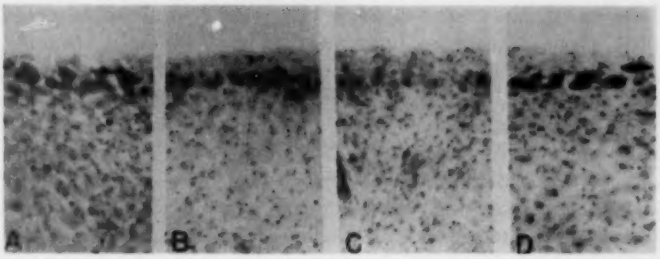

Fig. 4. — Sudanophilia of liver tissue after incubation for 24 hours in serum and *A*, oleate; *B*, stearate; *C*, palmitate; *D*, without additional fatty acid. The sections were counterstained with hematoxylin. All sections showed a marginal sudanophilia, but that incubated in serum oleate was maximal.

distribution of the crystals, whereas that which had been incubated in serum oleate showed only a moderate fluorescence.

Solubility Studies: The birefringent crystals resulting from incubation with stearates and palmitates disappeared completely after immersion for a few minutes in chloroform or ether. They were slowly soluble in 100% alcohol and acetone, disappearing entirely from sections only after three hours' immersion. They were not, however, appreciably soluble in 0.1 N NaOH, 0.1 N KOH or 0.1 N HCl (at room temperature for six hours) and were unaffected by prolonged immersion in neutral formalin solution.

General Observations: In comparison with cells which had been incubated in serum or serum oleate, those which had been incubated (or injected) with palmitate and stearate appeared somewhat foamy and had a granular cytoplasm. This was slight but definite and was evident in unstained as well as in stained sections.

It was also apparent that sodium palmitate and sodium stearate were less necrotizing when injected into the cornea than were comparable amounts of sodium oleate.

When sodium stearate or palmitate were added to a medium containing oleate the cells developed *both* a sudanophilic and crystalline lipid. In other words, the effects were additive and apparently independent. Nor was there evidence that the one inhibited the other in any way.

Liver.—Observations on the liver accorded with those on the cornea if allowance was made for the fact that liver buttons

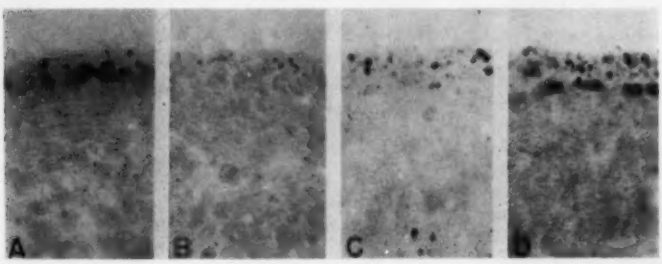

Fig. 5. — Osmic acid stain of liver tissue after incubation for 24 hours in serum and *A*, oleate; *B*, stearate; *C*, palmitate; *D*, without additional fatty acid. All sections showed some marginal take of stain, but that incubated in serum oleate was maximal.

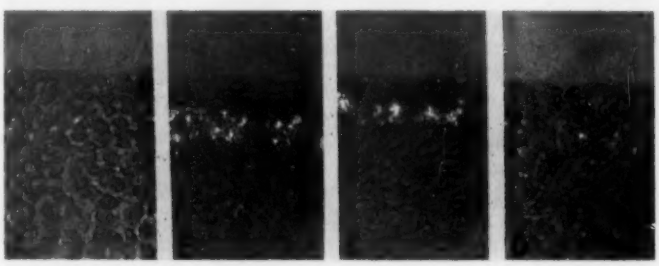

Fig. 6. — Birefringence in liver tissue after incubation for 24 hours in serum and *A*, oleate; *B*, stearate; *C*, palmitate; *D*, without additional fatty acid. Crystals were regularly found and relatively abundant near the margins of tissue incubated in serum stearate or serum palmitate but only sparse in tissue incubated in serum oleate or in unfortified serum.

show what has been called in previous publications a marginal lipogenesis (Figs. 4 and 5) to some extent even though the medium is not fortified with fatty acid.* Thus incubation of liver pieces in serum palmitate or serum stearate resulted in the marginal deposition of birefringent crystals, and these were absent or slight in liver buttons incubated in serum oleate or in serum alone (Fig. 6). Moreover, these crystal-containing cells have the same tinctorial features after incubation as those in the cornea and the crystals themselves have the same solubility properties.†

Comment

From observations on both cornea and liver, it appears that the cells of these tissues have the obligatory property of forming sudanophilic globules from an oleate substrate and birefringent crystals from a palmitate or stearate substrate. Although definitive identification of these end-products has not been accomplished as yet, they have the histochemical properties of both neutral fats and phospholipids. While these lipids result from exposure of the cells to appropriate fatty acids, they will not in turn induce lipogenesis when injected into other corneas. This is interpreted as indicating that their fatty acid content is bound in a form no longer available for neutral fat synthesis. In other words, the cells have not merely abstracted and concentrated the fatty acids from the medium but have synthesized a fat in which the original fatty acids have, as indicated by the radioactive studies, been incorporated. Studies of the end-products by chemical means, to be reported from this laboratory shortly, suggest that some at least are glycerides.[4,5]

Conclusions

Aberrant lipogenesis from palmitate or stearate substrate results in a nonsudanophilic crystalline product. This is the counterpart of the sudanophilic noncrystalline product of oleate origin. The histochemical and solubility properties of the lipids formed are compatible with the assumption that that induced by the oleates is a neutral oleate fat, possibly triolein and phospholipid, while that from palmitate and stearate is also a neutral fat, possibly tristearin or tripalmitin, respectively, and corresponding phospholipids.

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* This autogenous lipogenesis has been explained by the assumption that oleates are provided by the parenchymatous liver tissue.

† In interpreting these results it is important to differentiate the stearate- and palmitate-induced crystals from those which occur autogenously when liver buttons are incubated in serum. These latter are situated deeper in the buttons and spare the marginal zone; they are coarse and frequently dentritiform, and yet they, too, are lipid in nature, occasionally taking Sudan stain, and dissolve out in organic solvents.

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Primary Diffuse Amyloidosis of the Respiratory Tract

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Primary diffuse amyloidosis of the respiratory tract was first reported by Balzer.[1] It is to be contrasted with localized tumor-forming amyloid of the upper respiratory tract and with primary systemic amyloidosis. Primary systemic amyloidosis involves the lungs in about half of the reported cases,[2] but other organs also are affected. Localized tumor-forming amyloid of the upper respiratory tract, on the other hand, is rarely associated with amyloidosis of other organs.[3] The lesion is usually confined to the larynx. This form of amyloidosis sometimes is an incidental finding at autopsy. It may be asymptomatic and offers a relatively good prognosis after surgical removal.[4]

Primary diffuse amyloidosis of the lower respiratory tract differs from these two entities in several important respects. The site of involvement is the submucosa throughout the tracheobronchial tree. The larynx is not the predominant area involved, although it may be affected. The lesion often is symptomatic, producing obstructive bronchiectasis and chronic respiratory infection, but in contrast to osteochondromatosis of the lung,[4] the tracheal cartilages remain intact. Treatment by endoscopic removal of the tumor masses is sometimes carried out, with varying degrees of success.[5] Dystrophic cartilage and bone often form in the amyloid.

Primary diffuse amyloidosis of the respiratory tract is rare. Schottenfeld et al.[7] were able to collect 14 cases (including 1 of their own) in the world literature prior to 1951. Since then a number of similar cases have been reported.[6,8-12] The peculiar dystrophic bone formation sometimes associated with this entity has been discussed by Candiani [11] and Glauser,[12] but little progress has been made in determining the etiology of this unusual lesion.

The purpose of this report is to present a case of primary amyloidosis of the respiratory tract and to compare the pathological physiology which follows this lesion with that following another rare disease involving the tracheobronchial tree—systemic chondromalacia.

Report of Case

A white man was admitted to Butterworth Hospital June 3, 1957, and died June 6, 1957, at the age of 67 years. His admitting complaint was dyspnea. For 40 years his home was in Grand Rapids, Mich., where he was the proprietor of a dry-goods store. The patient had recurrent sinusitis, for which three operations were performed in childhood and early youth. At age 30, he developed paroxysmal wheezing dyspnea three or four times a year, each episode lasting from one to two weeks. These attacks developed after respiratory infections at any season and tended to be worse in the spring. Gradually these episodes became more frequent and forced his retirement 10 years before his death. He went to many medical clinics and moved to Florida and Arizona in search of relief. In July, 1955, he was examined at the University Hospital, Ann Arbor, Mich. Physical examination at this time disclosed that he was cyanotic and dyspneic at rest. The anteroposterior diameter of the chest was slightly increased, and the lateral thoracic veins were engorged. Marked clubbing of the fingers and toes was noted. Inspiratory and expiratory wheezing was present throughout both lung fields. X-ray examination of the chest showed extensive bilateral parenchymal abnormalities, with a honeycombed appearance suggestive of bronchiectasis. The diaphragms were flattened, and the costal vertebral angles were obliterated by pleuritis. The findings were considered indicative of pulmonary emphysema. Skin tests for blastomycosis, toxoplasmosis, and histoplasmosis were negative. The tuberculin test was negative in a dilution of 1:1000. Carbon dioxide-combining power of the blood was 69 vol. % and

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Department of Pathology, Butterworth Hospital.

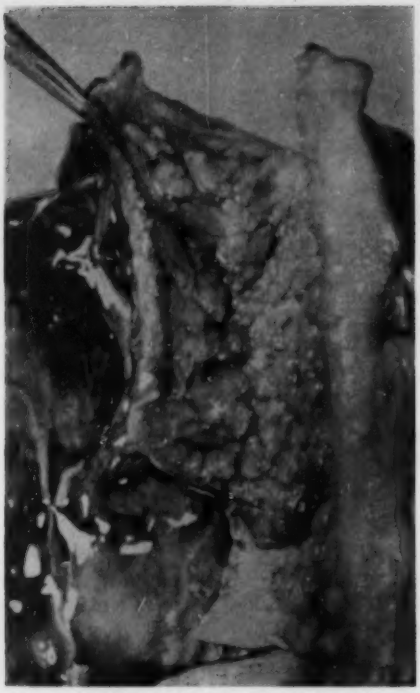

Fig. 1.—Irregular bosselations of amyloid projecting within the trachea.

74 vol. % on two occasions. Hemoglobin was 13.9 gm. %. The laboratory findings were otherwise negative. Therapeutic bronchoscopy was advised, but the patient refused the procedure, and he returned to Grand Rapids. Subsequent to this hospitalization he continued to be a chronic invalid. He was admitted to Butterworth Hospital severely ill two years later. On this admission the findings were substantially as described above except that the hemoglobin was 17.6 gm. % and cyanosis was extreme. The patient died on the third hospital day.

Autopsy Findings

Autopsy was performed three and one-half hours after death. Positive findings were confined to the cardiorespiratory systems. There was considerable coronary sclerosis, with dilatation and hypertrophy of the right heart. Both lungs were the site of striking bilateral bullous emphysema. The left pulmonary artery was occluded by firm thrombotic material. The most graphic lesions were present in the lungs and tracheobronchial tree. Throughout the entire respiratory tract, from the trachea to the terminal bronchioles, the mucosa was elevated by glassy bosselated irregular tan nodules (Fig. 1). These were confluent and projected from 3 to 5 mm. above the lumen of the tracheobronchial tree. In the smaller bronchi these bosselations completely encircled the bronchi and narrowed them markedly (Fig. 2). Some of the smaller bronchi contained purulent material. Cut surface of the pulmonary parenchyma disclosed numerous sac-like or cyst-like spaces.

Microscopic examination disclosed amorphous pink-staining material situated submucosally and producing small elevations of the overlying mucous membrane of the tracheobronchial tree. This material gave the characteristic metachromatic staining reactions of amyloid (Fig. 3). Within the collections of amyloid were small nests of cartilage and bone. The cartilage and bone did not proceed from the preexisting bronchial cartilages, which were demonstrated to be intact, but appeared to be arising within the amorphous amyloid material. The remaining pulmonary parenchyma showed a honeycomb appearance, with thickening of the alveolar septae and epithe-

Fig. 2.—Close-up of submucosal amyloid largely occluding bronchi.

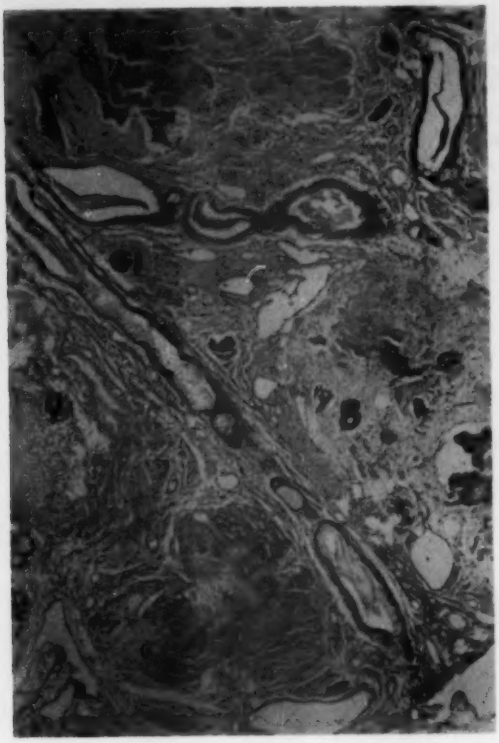

Fig. 3.—Amyloid deposits (*A*) within the submucosa of the trachea. Note the formation of cartilage and bone (*B*). Distorted submucosal glands are present (*Gl*). Hematoxylin and eosin; ×75.

lization of the alveolar lining. Areas of bronchopneumonia were present. Sections through the pulmonary artery disclosed the appearance of organizing thrombus. The remaining organs did not contain deposits of amyloid. No evidence of myeloma was identified.

Comment

It was considered worth while to record this case not only because of the rarity of the lesion but also because such infrequent diseases give additional information regarding the pathogenesis of commoner lesions. Review of similar cases previously recorded in the literature indicates that in a high percentage of instances chronic pulmonary disease with severe emphysema and bronchiectasis was present whenever the amyloidosis of the respiratory tract was sufficiently extensive to produce bronchostenosis. In contrast to this, the localized amyloidosis of the upper respiratory tract was not often associated with bronchiectasis and emphysema.

Another rare disease of the tracheobronchial tree may be contrasted to this entity. In systemic chondromalacia [13] the cartilage throughout the body, including the tracheobronchial tree, undergoes severe softening. The resultant collapse of the tracheobronchial tree tends to produce bronchostenosis and brings about spontaneous pneumothorax and emphysema. Such scattered observations made on rare diseases appear to indicate the association of processes which produce bronchostenosis with the development of some forms of emphysema.

Drs. A. J. Baker, Grand Rapids, and J. H. Sheldon, Ann Arbor, Mich., provided the clinical data. Dr. S. C. Capps, Grand Rapids, Mich., and Dr. A. A. Liebow, New Haven, Conn., furnished the illustrations. Dr. Liebow acted as consultant in arriving at the diagnosis and helped assess the significance of the case.

Butterworth Hospital (3) (Dr. Mann).

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Olfactory Esthesioneuroepithelioma

Report of a Case and Review of the Literature

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Olfactory neuroepithelioma is a rare tumor of adults, first described by Berger, Luc, and Richard in 1924. Mendeloff,[10] in reviewing the literature 33 years later, was able to collect 22 cases and added 6 personal cases.

Interest in this tumor is stimulated by its rarity and by its unique origin from the sensory cells of olfactory mucosa. To the histopathologist, it is important to differentiate esthesioneuroepithelioma from other tumors of the nasal cavity because of its striking radiosensitivity.[14] The case reported here is the only example of this tumor found in an active cancer hospital in a period of 13 years, during which time more than 40,000 specimens have been accessioned by the surgical pathology section.

Report of Case

The patient, a white man aged 74, was first seen by a physician in February, 1956, complaining of nasal obstruction and epistaxis of one month's duration. On examination, a large hemorrhagic friable tumor was found filling the right nasal cavity. It was apparently attached to the mucous membrane of the middle meatus. The tumor was excised transnasally, and the patient was referred to M. D. Anderson Hospital for further therapy.

At the time of admission, three weeks after initial excision, there was no physical or radiologic evidence of residual or metastatic disease. Because the diagnosis was in doubt, reexploration of the nose was carried out through a palatal approach, one month after the initial excision. Although no tumor was grossly apparent, the right turbinates were removed and the sphenoid sinus and ethmoid cells, exenterated. Microscopic foci of tumor were found in several locations in the removed tissue.

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Recovery from the operative procedure was uneventful. Five months later, recurrent tumor appeared in the nasal septum. At a second operative procedure the septum, the medial wall of the right antrum and the mucous membranes of the ethmoids and cribiform regions were removed. Tumor was histologically demonstrated in all regions except the ethmoids. Again recovery was uneventful, but six weeks after operation a cystic swelling appeared at the root of the nose; biopsy was done, and it was found to contain tumor.

A tumor dose of 500 r was given through a single anterior port and two lateral ports during five weeks ending Nov. 28, 1956. The mass at the root of the nose disappeared, and pain was relieved. Fifteen months after completion of therapy the patient was free of disease.

In sections of tissue removed on various occasions there are, among the normal structures of the respiratory epithelium and cartilaginous tissue, nests and sheets of tumor cells which in some areas are sharply marginated and in others blend gradually with the stroma which surrounds them. The tumor cells are oval or spindle-shaped, and they vary in size. Their nuclei are large and hyperchromatic, with irregular arrangement of chromatin and one or two clear nucleoli. Cytoplasm is scanty, pale, and eosinophilic.

In one of the sections, there are groups of epithelial cells—cubic, cylindrical, or poorly defined—with scanty eosinophilic cytoplasm and oval elongated dark nuclei, grouped in one or several rows, radially around a lumen forming the rosette-like structures typical of esthesioneuroepithelioma.

Comment

The olfactory area of the human extends from the middle of the roof of the nasal cavity 8 to 10 mm. downward on each side of the septum and on the surface of the superior turbinates. It consists of pseudostratified nonciliated columnar epithelium

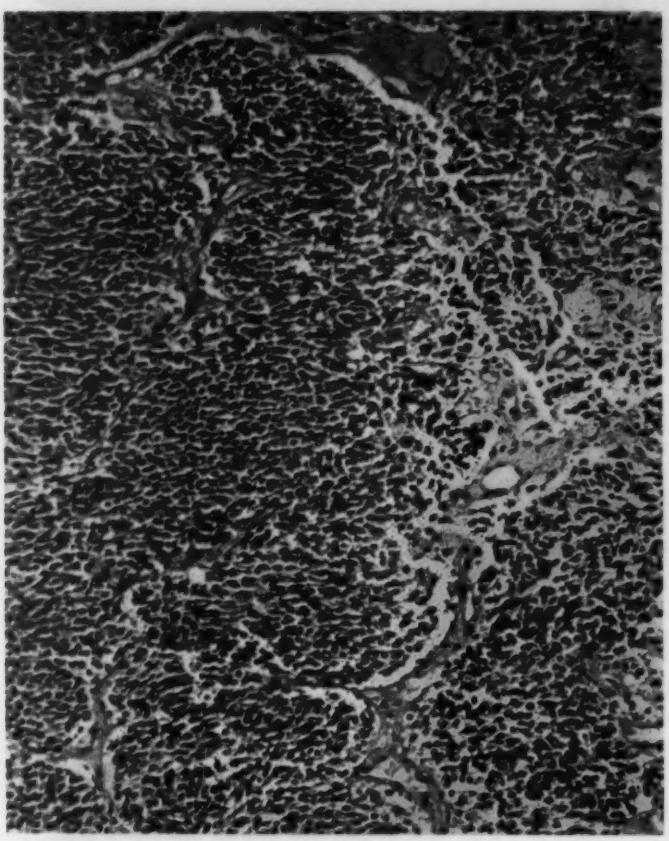

Fig. 1.—A section from the tumor, showing diffuse growth. This pattern predominated.

which lacks a distinct basement membrane. Isolated areas of similar epithelium are present in the anterior ethmoid cells and in scattered areas throughout nasal mucosa. These structures, which in aggregate cover 250-500 sq. mm., constitute the "olfactory placode" of the French authors.

Olfactory epithelium consists of supporting cells, basal cells, and olfactory cells.[9] The supporting cells are columnar, with an axial bundle of tonofibrils. Each cell presents a cuticular plate at its free surface and contains a diplosome and a tiny flagellum. The basal cells form a single layer of small conical elements at the base of the supporting cells and have dark nuclei and branching processes. The olfactory cells are bipolar neuroepithelial cells which lie between the deep processes of the supporting cells. From the distal pole of each olfactory cell, a thick straight cylindrical process emerges and passes through small openings between the supporting cells to the surface, where it projects as a hair-like process, the "olfactory hair." From the base, a slender tortuous nonmyelinated fiber emerges, covered by neurilemma. Proceeding centrally, it joins with other similar processes to form a nerve bundle. There are about twenty of these "fila olfactoria." They pass through the openings of the cribiform plate of the ethmoid bone and enter the substance of the olfactory bulb of the brain.

Tumors originating from this complex structure have been reported only 20 times in the American literature, although several other less well-documented cases are mentioned by various authors.[1-3,11,15] Patients have ranged from 8 to 79 years of age; there is no predilection for either sex. The main

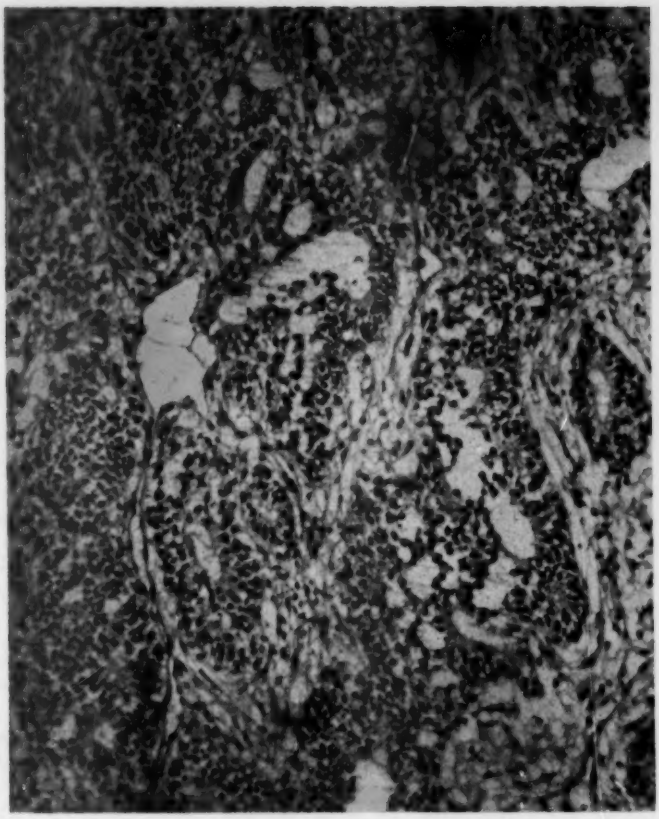

Fig. 2.—An area showing rosette formation.

symptoms are of progressive nasal obstruction and hemorrhage. Onset is usually insidious, and pain, absent. Local destruction of the bones of the nose and orbit has been observed in only one case, late in the course of the disease. Lymph node metastases are very rare. Recurrence has been almost invariable following surgical removal.

Like tumors arising in neuroepithelium elsewhere, olfactory esthesioneuroepithelioma is characteristically highly radiosensitive. In all the reported cases treated by radiation the tumors regressed completely for periods ranging from six weeks to eight years. One patient had four recurrences in a 20-year period, each responding to radiotherapy. Numbers of patients are too few and observation too incomplete to permit a statement that these tumors can be cured by radiation.

Grossly, olfactory esthesioneuroepitheliomas are large polypoid masses of friable vascular tissue usually attached to the roof of the nasal cavity.

Two different histological structures have been described.[3,6] Either may occur singly, or they may appear concurrently in different areas of the same tumor, or the two patterns may be diffusely intermingled. In one pattern, groups of cylindrical cells are arranged around a lumen, forming typical rosettes. In the other, cords and sheets of undifferentiated cells with abundant cytoplasm and indistinct cell walls are found, reminiscent of retinoblastoma or neuroblastoma.

Berger and Coutard thought the cells forming the rosettes arose from the bipolar nerve cells of the olfactory sense organ. If both structures were present they called the

tumor "esthesioneuroepithelioma," but if the rosettes were absent, they designated it "esthesioneurocytoma." In Seaman's case, the histological picture was of abundant closely packed tumor cells with scanty cytoplasm and small homogeneous blue-staining nuclei with poorly defined nucleoli, supported by abundant connective tissue stroma. There was no evidence of rosettes or reticulum being formed by the tumor cells. It coincided with the "esthesioneurocytoma" of Berger and Coutard. In the case described here, the tumor is also predominantly of the undifferentiated pattern.

In the past there has been considerable confusion between esthesioneuroepithelioma and other tumors growing in the nose,[15,17] especially those of neural origin. Foremost of the lesions requiring differentiation is the intranasal fibroglioma, a benign slowly growing congenital tumor, histologically composed of islands of glial tissue embedded in vascular fibrous tissue. Anglade and Philip thought such tumors originated from the heterotopic neuroglial cells demonstrated by Ramón y Cajal to occur in nasal mucosa.

Nasal encephaloceles have frequently been mistaken for both olfactory esthesioneuroepitheliomas and fibrogliomas.[16] Encephalocele is not a neoplasm but arises in fetal life as a bud from the anterior cerebral vesicle and protrudes into the nasal cavity, interfering with closure of the membranous skull. Lesions may be formed ranging from a cyst lined by ependyma, filled with cerebrospinal fluid, and communicating with the ventricular system to a solid mass of glial tissue entirely separate from brain.

Other tumors of neurogenic origin, such as ganglioneuroma, neurilemmoma, and nasopharyngeal fibroma occur rarely in this region and may be confused grossly with olfactory esthesioneuroepithelioma. Histologically, olfactory esthesioneuroepithelioma must be distinguished from undifferentiated carcinoma or lymphosarcoma.

Summary

This report describes a case of a patient with an olfactory esthesioneuroepithelioma which was excised three times with recurrence after each excision. After radiotherapy the tumor regressed completely, and the patient has remained free of disease for 15 months.

Department of Pathology, M. D. Anderson Hospital (25) (Dr. Gallager).

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Regeneration of the Fundic Mucosa in Rats

II. Effect of Various Hormones and of Ablation of Endocrine Glands on Epithelization

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In a previous publication,[1] healing of experimental defects in the fundic mucosa in rats was found to be uninfluenced by estrone administration as well as by castration in either sex with regard to epithelial covering and development of new glands.

The aim of the present article is to study the effect of some other endocrine activities in this respect, including administration of testosterone, cortisone, adrenocorticotropic and growth hormones, thyroid hormone, and I^{131}, as well as hypophysectomy and adrenalectomy. Epithelial regeneration alone will be dealt with in this study.

Materials and Methods

The material and the experimental technique were similar to that previously described.[1] Artificial wounds were produced in the anterior wall of the fundic mucosa of the rat stomach while the animals were subjected to the various hormonal influences.

According to the conclusion from the first article, complete epithelial covering may be expected under normal conditions from approximately the 14th day. The 13th and the 15th days, therefore, were chosen for observation. Further, observations were made in surviving animals on the 21st and the 180th days. Each group comprised eight adult rats of the same strain as before, four males and four females. They were kept on the standard diet of our laboratory, four animals in each cage.

The hormone injections started one week before the gastric operation was performed and lasted until the 30th day postoperatively. The ablation of endocrine glands was also performed one week before the gastric operation. The animals were killed with ether, and the stomach was immediately removed for fixation in formalin. Sections of the stomach were taken out according to the scheme previously described.[1] The body weight of animals was noted during the experiments. At autopsy the weight of the pituitary, testes, prostrate gland, uterus, ovaries, adrenals, kidneys, and liver was registered in the majority of animals. Sections from these organs and from the spleen were taken out for microscopic control.

Dosage.—The hormone doses were estimated in proportion to the relation between weights of humans and rats. The ratio 300:1 corresponds to a human weight of 70 kg. and a rat weight of 233 gm. As different animals may respond differently to various hormones,[2] the general effect of the hormones was tested in different ways.

Testosterone.—Testosterone (Testin, Nyco) acetate was given in daily doses of 0.5 mg., corresponding to 150 mg. in humans. The weight of the prostate gland was significantly (67%) higher in the treated group than in the control group.

Cortisone.—Cortisone (Cortone, Merck) acetate was injected daily in doses of 0.3 mg., corresponding to 100 mg. in humans. These injections resulted in a considerable (17%) decrease in weight of the adrenal glands.

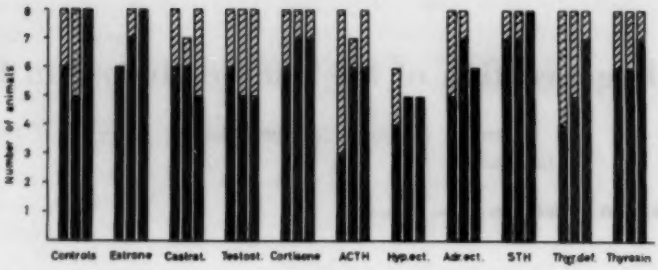
Adrenocorticotropic Hormone.—Corticotropin (Jaton Prolongatum, A. L.) was administered in daily doses of 0.13 I. U., corresponding to 40 I. U. in humans. This treatment was tested in an unoperated-on group of 10 animals by means of the ascorbic acid depletion method (Sayer). The results of these tests were not convincing, though some animals showed a distinct effect.

Growth Hormone.—Somatotropin (Somacton, Nordiska Hormonlaboratoriet AB) was given in daily doses of 5 tibia units equivalent to 0.25 mg. The preparation used does not contain corticotropin (ACTH). The size of doses is still under discussion. The dose used here corresponds to 1 mg. per kilogram of body weight per day. This has been used in animal experiments as the smallest diabetogenic dose.[3] No parallel tests with regard to growth-promoting action in young animals were performed in this series.

Thyroxin.—Thyroxin (Roche) was injected in daily doses of 0.02 mg., corresponding to 6 mg. in

Submitted for publication June 9, 1958.

From Institutt for Patologisk Anatomi, Rikshospitalet; Chief, Professor Olav Torgersen, M. D.



Epithelial covering of defects following gastric operation in animals subjected to various endocrine influences. The three columns in each group represent relative covering on the 13th, the 15th, and the 21st day, respectively. The solid part of the columns represents animals with full covering of the defect. (The first three groups represent results reported previously.)

humans. The animals of this group lost weight during the week from the initial hormone injections until the gastric operation, while the animals in all other groups gained weight during the week prior to the gastric operation.

Hypophysectomy.—Hypophysectomy was performed by Smith's parapharyngeal method as modified by Böe (unpublished). The material removed was examined microscopically to evaluate the effectiveness of the operation, and the hypophyseal region was further examined at autopsy. Fifty-three animals were used for this series, but only sixteen are included in the material. The others had to be discarded, owing to incomplete removal of the pituitary, or they died following the hypophysectomy.

Adrenalectomy.—Bilateral adrenalectomy was performed by transabdominal approach. The first five animals, which got no substitution therapy, died in the course of 24 hours. Of the next 32 animals, 24 survived. These got an injection of 2 mg. cortisone on the day of adrenalectomy and 1% salt water to drink until they were killed.

Thyroid Deficiency.—Thyroid deficiency was induced by administration of radioiodine. A single dose of 0.3 mc. was given intraperitoneally, which results in thyroxin deficiency lasting for at least one month.[4] Microscopy of the thyroid glands revealed severe degenerative changes in all animals of this group.

Various Hormonal Influences

In the following paragraphs dealing with various hormonal influences some general and special aspects are considered regarding tumorigenesis, cytology, secretion, and regeneration.

The findings in the first report [1] are in agreement with results published subsequently by other authors,[5] who found no effect of estrogen on formation of tannic acid (tannin)-induced ulcers in the rat stomach.

Testosterone. — Several observations in the literature point to sex hormones as possible regulators of regeneration of the gastric mucosa.[1] The predominance of males among patients with gastric ulcer and cancer and the high incidence of gastric cancer in males over 50 years of age compared with the equal sex distribution among patients under 40 years of age [6] may favor this view. Nearly all neoplastic changes occur in a period when hormone changes take place.[7]

It is a well-established fact that sex hormones are of importance to neoplasms in other organs than the stomach, and an antagonism between estrogen and androgen with regard to carcinogenic effects has been suggested.[8-11] These suggestions have found successful clinical use in the treatment of mammary and prostatic cancer.[9]

Experimentally gonadectomy showed no effect on the gastric zymogenic cells.[12]

Corticotropin and Hypophysectomy. — Summarizing the role of the pituitary in tumorigenesis, Twombly and Pack [13] conclude that there is no definite evidence of any direct action of pituitary hormones on tumorigenesis. It is well established, however, that through its effect on the steroid-producing glands the hypophysis may exert an important indirect effect upon the growth of tumors and on the condition of the host tissue.

Numerous experimental and clinical reports on the influence of the pituitary hormones on function and cytology of the stomach have been published. Increased output of hydrochloric acid in dogs [14] and

humans [15] is reported, and decreased volume of gastric secretion has been demonstrated after hypophysectomy.[16] Reduction of pepsin secretion as well as severe involution of zymogenic cells have been found after hypophysectomy.[17] Some observations [12] suggest that the secretory activity of the gastric zymogenic cells is affected by the hypophysis through the thyroid gland and the adrenal cortex, the latter route being most important.

Atrophy of the gastrointestinal tract is reported after hypophysectomy,[18,19] while some authors [13] consider that the hypophysis determines only the proportional increase in size of the body as a whole, whereas growth of individual cell groups is less affected.

There seems, however, to be general agreement with regard to the inhibiting effect of corticotropin on formation of granulation tissue. Delayed healing was found in experimental abdominal wounds in rats following cortisone and corticotropin administration,[20] while neither cortisone nor corticotropin seemed to prevent healing of experimental gastric lesions.[21] A recent paper [22] records the interesting findings that corticotropin and cortisone did not seem to interfere with the replacement of mucus epithelium in corpus pouches following chemical desquamation. The healing of excision ulcers in gastric explants, however, was clearly retarded in dogs. No change in the rate of epithelization of skin wounds or of fibroplastic repair of stomach wounds in the rat following hypophysectomy have been reported.[23] Delay in regeneration of the gastric mucosa in hypophysectomized rats has been reported recently.[24]

The development of or aggravation of ulcer symptoms in patients treated with corticotropin has been explained by the stimulating effect of corticotropin on gastric secretion associated with retarded healing.[14]

Growth Hormone (Somatotropin).—It is evident from animal experiments that the hypophyseal growth hormone [25] has a growth-promoting effect,[26] and it is considered to have a beneficial effect on wound healing.[27] Hypophysectomized rats continue to grow under somatotropin administration.[28] Hyperplasia and malignant tumors develop in rats under continuous somatotropin administration.[29]

Cortisone and Adrenalectomy.—Conflicting reports with regard to the influence of adrenal cortical hormones on gastric function and cytology are likewise found in the literature. Thus, some authors have found increased gastric secretion under corticotropin and cortisone administration,[15] while others have reported decreased acid secretion under cortisone administration.[30] Still other authors [31] claim that the hormones of the adrenal cortex play no direct or important part in the acid secretion mechanism. Finally, some investigators state that various secretory functions, including gastric secretion, are influenced by adrenal cortical hormones.[32]

Decreased gastric secretion following adrenalectomy has been reported by various authors.[12,33] The results of the former may indicate hypophyseal regulation of zymogenic cells via the thyroid and the adrenal cortex. Histologic observations are in conformity with biochemical data showing a stimulating effect of adrenocortical hormones on gastric secretion.[34] Decreased acid secretion after adrenalectomy has been restored by administration of adrenal cortex extract [32] and cortisone.[30]

Corticotropin and cortisone are considered to have an inhibitory effect on wound healing,[35] which has been demonstrated in experimental abdominal wounds.[20] However, neither cortisone nor corticotropin showed a "regularly harmful" action on experimental gastric lesions.[21] Accordingly, no influence on the epithelial healing was found in experimental gastric ulcers by cortisone administration in spite of inhibitory effect on fibroplasia.[36] These findings harmonize well with a paper published recently.[22] Delay in the development of all connective tissue elements was found in rabbits after large doses of cortisone.[37] Cortisone also reduces the rate and extent of vascularization in rabbit ear chambers.[38] Adrenalectomy causes de-

creased weight of the mucosa in all regions of the gastrointestinal tract [19] and delay of regeneration of the gastric mucosa.[24]

Cortisone injections have been shown to inhibit the rate of growth of the Ehrlich ascites tumor in mice.[39]

Hypophysectomy and adrenalectomy do not prevent the growth of experimental tumors in animals, but it may be delayed, or the number of takes may be diminished.[35]

Thyroid Gland.—It is generally accepted that the thyroid hormone effects the growth, maturation, and differentiation of tissue. It could therefore be reasoned that an excess of thyroid hormone might facilitate development of cancer and that a deficiency of the hormone might inhibit carcinogenesis. From the conflicting results reported it is readily apparent that the role of the thyroid in cancer development is still obscure.[40] The correlation between the thyroid and neoplasms has been investigated, however, with rather unconvincing results. The functional state of the thyroid does not seem to have any influence on the development or growth of experimental or human cancer. The coincidence of hyperthyroidism and thyroid cancer is rare.[13]

Extensive data support the importance of local factors being responsible for known local variations in the incidence of thyroid cancer. The supply of iodine, traced by the goiter incidence, seems to be such a factor. Clinical observations together with perusal of experimental studies give additional support to these observations.[41] Most interesting is the view that compensatory hypersecretion of tropic hormones caused by hyposecretion of target cells may be a cause of cancer.[42]

Thyroidectomy or treatment with I^{131} or thiouracil may stimulate the growth of well-differentiated thyroid cancers or may even convert them into anaplastic carcinomas. It is probably not the radiation from the I^{131} but the secondary hypothyroidism and the increased output of thyroid-stimulating hormone that stimulate the cancer.[43]

Total thyroidectomy by I^{131} injection significantly prolonged the time interval between methylcholanthrene injection and the time of development of sarcoma.[40]

Thyroidectomy does not change the cytology of the stomach but reduces the volume of gastric juice after pyloric ligation.[12] Increase of thyroid hormone curtails, while decrease prolongs, the time of healing of skin wounds in animal experiments.[44]

It appears that radioiodine may be used to effect total thyroidectomy in the rat without appreciably deleterious side-effects. An amount of 0.875 mc. of I^{131} has been found adequate for complete destruction of the thyroid in rats.[45] The cytological changes in the anterior pituitary are identical with those occurring after total surgical thyroidectomy.[4] Evidence has been presented to show that the changes in the pituitary are not the result of radiation damage to the pituitary but solely the consequence of thyroid destruction.[4] Examination of the pituitary after injection of 0.3 mc. of I^{131} revealed that these animals have a thyroxin deficiency at least for one month after the I^{131} injection.[4] A very different matter is the development of carcinoma of the thyroid one and one-half to two years later [46] after injection of 0.4 mc. of I^{131}. Taking into consideration the hypothesis of cancer development caused by hypersecretion of thyrotropic hormone due to thyroid deficiency, it is interesting to note regression of thyroid cancers following administration of desiccated thyroid.[43]

Results

The microscopic criteria used to estimate the findings include the rate of epithelial defect covering, the formation of new gastric glands, types of cells, mitoses, dilatation of glands, and diastasis. Some other observations were also noted.

The degree of epithelial covering is summarized in the Figure, which shows the number of animals in each group revealing full covering of the defect on the 13th, 15th, and 21st days, respectively, after gastric operation.

The main conclusion is that from the 15th day and onward there is no significant difference between the various groups with

regard to full epithelial covering. A tendency exists toward retardation in some groups before that time, which is the case in the corticotropin-treated and the I^{131}-treated groups. These two groups, however, like all other groups, behaved in the same manner with regard to epithelial regeneration from the 15th day after gastric operation. The somatotropin-treated group revealed the highest number of animals with full covering on the 13th day. Further, no particular difference in the degree of new gastric gland formation was found between the various groups. Neither did the estimated criteria reveal any difference between the two sexes in any respect.

The number of artifacts caused by large diastasis ranged from zero to five in the different groups. Exclusion of these artifacts would have led to still better conformity between the groups, but it is my opinion that exclusion would be misleading, as diastasis might indicate a tendency to decreased strength in the affected wounds. The largest number of diastasis was encountered in the testosterone-treated group, while the corticotropin- and cortisone-treated groups revealed one and no diastasis, respectively.

The majority of animals in all groups showed dilated glands. Severe infection was found in some animals. However, the abscesses were lying outside the gastric wall and did not seem to interfere with the epithelial healing. Most abscesses were found in the testosterone-treated group, which showed the usual tendency of healing.

Comment

The results of the experiments presented in this paper indicate that epithelial regeneration of artificial wounds in the glandular stomach in rats is uninfluenced by various hormonal imbalances produced in this study.

Objections may be raised against these experiments because of the size of the doses. As mentioned before, however, the main principle has been to give the animals doses of hormones corresponding to doses administered clinically to humans. The doses have been calculated according to the respective weights and have been estimated near the upper limit of human doses. The strict relationship between doses in humans and animals in proportion to body weight is not a matter of course. One point is that the quantitative relationship between the amount of a hormone administered to an animal and the response produced varies in different animals and even in the same animal at different times.[2] It is emphasized that it has not been the intention of these experiments to give large doses for the mere purpose of getting an outcome. This principle must have been abandoned, of course, in connection with ablation of endocrine glands in order to establish negative hormonal balance. It is shown for each hormone in the above paragraph on dosage that the doses used really have a general effect on the animals.

However, it seems very likely that the dosage would be of importance for the healing of the defects, though this is not quite sure. Other authors [21,36] have published results which are in accordance with those reported here, using twice or three times higher doses of cortisone, for example, a hormone which is generally accepted to inhibit formation of granulation tissue and to retard wound healing. That the delay in healing of gastric wounds in dogs was not in direct correlation to the dosage of the drug employed is emphasized by Janowitz et al.[22] The fact that even the greatest hormonal deficiencies do not affect epithelial wound-healing under the procedure employed in these experiments is exemplified by such radical procedures as hypophysectomy and adrenalectomy.

The findings in this study tally with those in several reports in the literature. It is worth mentioning that the results concerning healing of experimental gastric lesions following corticotropin and cortisone administration are consistent with the findings of Rodriguez-Olleros and Galindo [21] and of Williams.[36] As far as epithelial healing is concerned, this study also agrees with the

recent findings of Janowitz et al.,[22] who found, however, retarded healing of experimental ulcers in dogs, as did Skoryna et al.[24] in rats, both using much higher doses of cortisone. Thus, not all findings in the literature tally very well with the generally accepted concept of an inhibitory effect of these hormones on the formation of granulation tissue and with the numerous reports of the influence on gastric function and cytology.

The lack of changes in healing capacity following hypophysectomy and adrenalectomy, inducing involution of gastric cells and decreased weight of the mucosa, as reported by Friedman[18] and Haeger,[19] is possibly contradictory. Skoryna et al.[24] found delayed regeneration in gastric lesions after hypophysectomy and to a smaller extent in adrenalectomized rats. However, Mueller and Graham's observations of the healing of skin and gastric wounds after hypophysectomy[23] are in agreement with the results obtained in the present study.

Although practically every facet of normal physiology is under some sort of control by one or more hormones and although hormones often play an important part in several pathologic conditions, these experiments seem to demonstrate that they have minor, if any, influence on epithelization in experimental gastric lesions in the rat. Normal state and optimal function of a cell is presumed to be the outcome of the interplay between several hormonal and nutritional factors, as is also true of most metabolic processes.[47]

It is my opinion that, according to the general view, the lack of findings in the corticotropin treated group, for instance, is strange. Objections may be raised against the size of the groups and of the dosage, though others have shown that the usage of higher doses does not always result in retarded healing. Nevertheless, in an attempt to solve this problem, a new series of experiments will be carried out with higher dosage and more animals. The behavior of the connective tissue will be dealt with in a subsequent paper.

Summary

The effect of various hormonal influences on regeneration of experimental defects in the glandular stomach has been studied in rats, including administration of testosterone, adrenocorticotropic and growth hormones, cortisone, thyroxin, and I^{131}, as well as hypophysectomy and adrenalectomy.

No significant difference was found in the various groups with regard to epithelial covering of the defect and development of new gastric glands. All groups revealed the same rate of epithelial covering from the 15th day and onward.

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Mycetoma of the Hand

Report of a Case

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Mycetomas, chronic granulomatous mycotic tumefactions with or without the formation of draining sinuses, rarely occur on the hand. In 1954, Moore[1] reviewed 20 cases of mycetoma of the hand and/or arm from the world literature and added a case of his own. Of these 21 cases, 5 were reported from the United States. Since the majority of the cases in this review were due to actinomycetes or true fungi producing dark granules grossly, the occurrence of a mycetoma of the hand due to a true fungus forming white-yellow granules warranted this report.

Report of Case

A 69-year-old married woman from Tarboro, N. C., was admitted to her local hospital because of nausea and vomiting associated with right upper quadrant pain.

In 1946 the left breast was resected because of carcinoma; at this time it was noted that the patient had mild diabetes mellitus. Several months later she received 5000 r to the left chest. A cholecystectomy was performed in 1951.

For the previous year the patient had been on insulin and digitalis.

The physical examination revealed a 1×1 cm. hard nontender freely mobile nodule on the dorsum of the left hand at the distal end of the fourth metacarpal. The remainder of the examination was within normal limits.

The course in the hospital was uneventful; the nausea improved when the digitalis was withdrawn and the diabetes controlled. An intravenous gallbladder study was negative. The nodule on the left hand was removed *in toto* as a possible metastatic nodule of the former breast cancer and sent to the Pathology Department of the Bowman Gray School of Medicine for histological study. A roentgenogram of the left hand was negative for extension and bone involvement.

Submitted for publication June 3, 1958.

From the Department of Pathology of the Bowman Gray School of Medicine of Wake Forest College and the Laboratories of the Baptist Hospital.

Pathology

Macroscopic Examination

The specimen submitted was an irregular lobulated nodule of firm slightly resilient gray-brown tissue which had been previously fixed in a solution of 10% formalin. The specimen had been previously sectioned and in this condition was 1.7× 1.2×0.6 cm. in its greatest dimensions. The sectioned surface was likewise firm and gray-tan, with numerous small discrete yellow-white nodules; the largest of these was approximately 0.3 cm. Although the tissue was quite firm, the yellow-white nodules were easily forced from the tissue and were then noted to be friable. There was no capsule.

Microscopic Examination

The discrete yellow-white granules are composed of masses of interlacing hyphae which under high power are segmented and branched (Fig. 1). The hyphae radiate toward the periphery of the granule, where they terminate as large chlamydospores. A dense exudate composed of granulocytes surrounds the granules; this in turn is surrounded by a granulomatous process within which there are numerous foreign-body type giant cells and large macrophages (Fig. 2). Each granule is then surrounded and separated from each of the others by a zone of fibrous connective tissue. All the granules are confined within the fibrous tissue, and no abscess appears to open onto the surface of the lesion.

Comment

The patient noted the lesion 18 months previously as a slowly growing nodule but gave no history of trauma to or drainage

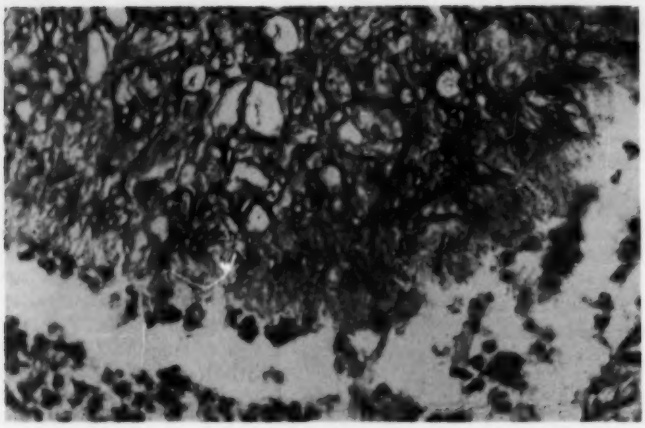

Fig. 1.—Edge of granule, showing hyphae and chlamydospores. Periodic acid-Schiff stain; × 480.

from the affected area. During this time medical therapy was never specifically directed to the nodule.

Since the nodule was well circumscribed, removed *in toto*, and shipped in a preservative, culture of the original site or of the excised nodule was impossible. The fact that this was a mycetoma with white-yellow granules offered the possibility that Allescheria boydii (Monosporium apiospermum) was the etiological agent. The patient's hobbies of gardening and chicken-raising lend some support to this conjecture, since this organism had been isolated from the soil in the United States by Emmons [2] and Ajello.[3] There is a strong possibility, however, that the etiological agent could be one of the other numerous organisms known to cause mycetoma, since it is known that the color of the granule is an unreliable criterion for the identification of the causative agent.

This was the second case of mycetoma encountered in this department. The first case was seen by Dr. R. P. Morehead,[4] in 1947, and represented a true fungus myce-

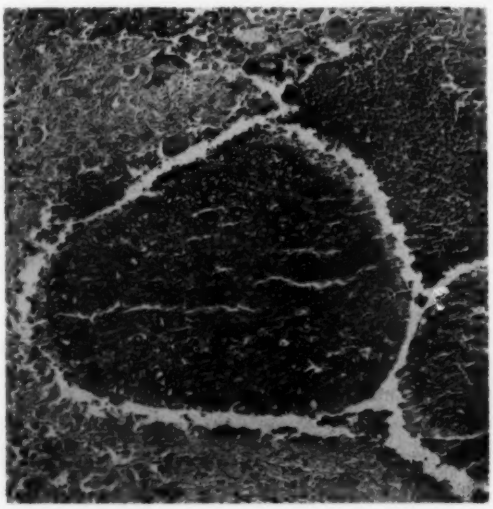

Fig. 2.—Granules surrounded by a nonspecific and granulomatous reaction. Periodic acid-Schiff stain; × 142.

toma of the foot proven by biopsy; the material was not cultured.

Summary

A case of mycetoma of the hand caused by a true fungus forming white-yellow granules is presented; the original site or the excised nodule could not be cultured.

Dr. Lewis Thorp and Dr. E. S. Boice provided the history of the case and gave us permission to present it.

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Lymph Node Structure and Metallophilia in Tumor-Bearing Mice

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Within recent years there have been numerous reports of immunological reactions induced by the implantations of various experimental tumors into different hosts.[1,2] While such data are interesting and provocative, it is not clear whether they are pertinent to the problem of spontaneous cancer in man or in animals. As pointed out by Graham and Graham, "The demonstration of immunological host-tumor differences in humans has never been very convincing." [3]

Recently Hirsch et al. investigated the ability of inbred mice to be immunized against their own tumors.[4] They found no differences in the total number or time of appearance of the tumors between the experimental and control groups. However, the "immunized" group did seem to have a small but significant increase in survival time, as compared with the controls. In view of the subtle nature of immunological reactions in the spontaneous tumor host, it seemed appropriate to approach the problem from another direction, namely, an examination of the regional lymph nodes for evidence of reactivity of the type associated with antigenic stimulation.

It has been demonstrated that antigenic stimulation in man and experimental animals leads to follicle formation and plasma-cell proliferations in the regional lymph nodes.[5] In addition, we recently reported that antigenic stimuli produced well-defined changes in ammoniacal silver staining of lymph nodes.[6] If the tumor-host relationship does involve antigen-antibody reactions of the hyperimmune type, then the regional lymph nodes would be expected to show such reactive changes.

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Material and Methods

An investigation was made of the structural and cytological features of the axillary and inguinal lymph nodes from 43 CFW mice bearing spontaneous mammary carcinomas, 27 adult control CFW mice, and 10 CFW mice bearing implanted fragments of spontaneous breast carcinoma from CFW mice. The lymph nodes were removed immediately after the mice had been killed by rapidly crushing their cervical cords. After the skin was laid back, the axillary and inguinal regions were flooded with 10% neutral formalin. The axillary, brachial, and inguinal lymph nodes were then carefully removed and fixed in 10% neutral formalin. Routine paraffin sections (5 mμ) were prepared, and duplicate sections were stained with hematoxylin and eosin (H & E) and with ammoniacal silver. The ammoniacal silver staining was performed as reported previously.[6]

Results

The H & E and the ammoniacal silver staining methods revealed a variety of structural and staining characteristics among the lymph nodes from different mice. Nevertheless, it was possible to group the cases according to relatively distinct features. Such subdivisions were suggested by our previous studies of lymph node reactions to antigenic stimulation.[6] The designation and the characteristics of the various patterns are as follows:

Control.—This lymph node pattern corresponded in structural features and staining reactions to the pattern which was found most frequently in lymph nodes from adult control mice. Follicular prominence and plasma-cell aggregates were minimal to

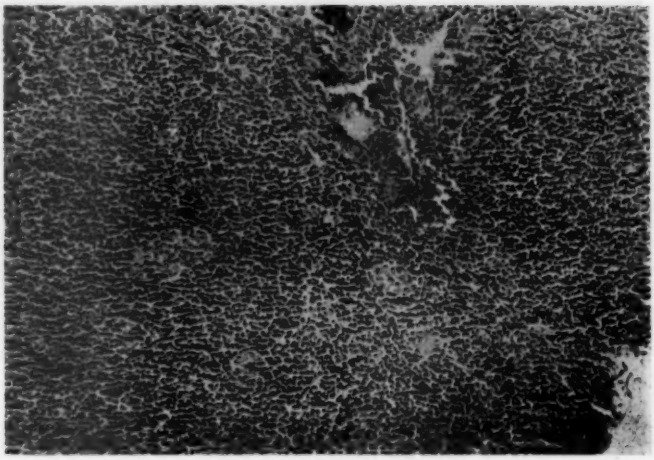

Fig. 1.*—Control pattern, H & E stain.

moderate. The sinusoids varied in size and cellular content. Silver staining revealed spider-like reticular cells in the pulp, while the littoral cells and sinus phagocytes stained with moderate intensity (Figs. 1 and 2). This appearance corresponds to the metallophil type of staining reaction described by Marshall.[7]

Antigenic Reactivity Patterns.—This heading includes four or five patterns which correspond to sequential phases, which appear in the response to antigenic stimulation, i. e., foreign protein.

Nonimmune Phagocytosis.—This pattern may or may not be seen after antigenic stimulation. It is characterized by an accentuated type of metallophilic stain of the pulp reticular cells as well as the phagocytic cells in the lumen of the sinusoids. In the present study we have included this pattern with the controls.

Recognition.—This type of change has been seen within hours of antigenic stimulation. The structure of the lymph node (H & E) is essentially similar to that of the control type. However, the silver technique yields a distinct nuclear stain of the lymphoid cells. The appearance of the section resembles an iron-hematoxylin prepara-

* Figures 1-13 depict various lymph node patterns encountered in axillary lymph nodes of CFW mice.

Fig. 2.—Control pattern, ammoniacal silver stain. Note silver staining of cytoplasmic processes of the spider-like metallophil cells scattered throughout pulp. Lymphocytes are not stained.

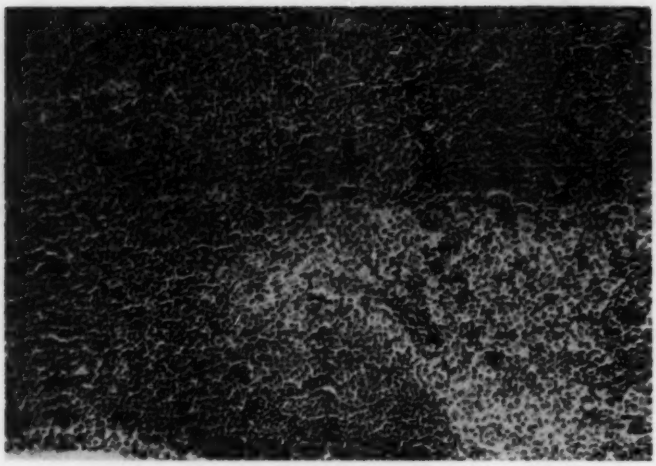

Fig. 3. — Recognition pattern, H & E stain. The appearance is essentially similar to that of the control pattern.

tion (nuclear type stain) (Figs. 3 and 4). The reticular and sinusoidal cells are usually stained poorly or not at all.

Reactive.—This pattern is characterized by the presence of prominent follicles with secondary centers and plasma-cell aggregates. The silver staining is a strong nuclear type (Figs. 5 and 6).

Immune.—The microscopic structure as seen after H & E staining is similar to that of the *reactive* type. However, the plasma cells tend to undergo degenerative changes. Typically, the sinusoids are dilated and filled with many macrophages. After silver staining, the appearance resembles an altered *control* type of metallophil stain. The reticular cells of the pulp, the littoral cells, and sinus phagocytes are strongly stained. Most characteristic is the presence of rounded hypertrophied metallophil cells in the follicles, which resemble gitter cells of the brain (Figs. 7, 8, and 9).

Exhausted.—The appearance after H & E staining resembles the *immune* or *reactive* type except that degenerative changes are seen in the plasma-cell aggregates and sinusoids. Silver staining reveals only poor staining of all elements, with smudging of the staining in the sinusoidal regions (Figs. 10 and 11). This pattern seems to be as-

Fig. 4. — Recognition pattern, ammoniacal silver stain. Note the strong nuclear type of staining and loss of metallophil type of staining. Contrast with control pattern after silver staining.

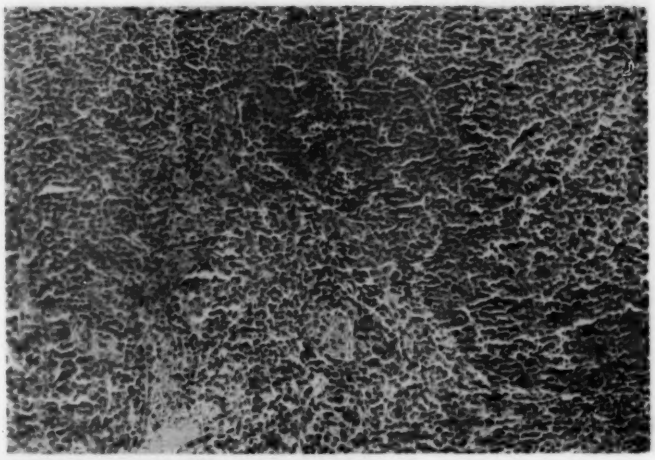

Fig. 5.—Reactive pattern, H & E stain. Follicle formation and plasma-cell aggregates (medulla) characterize the picture.

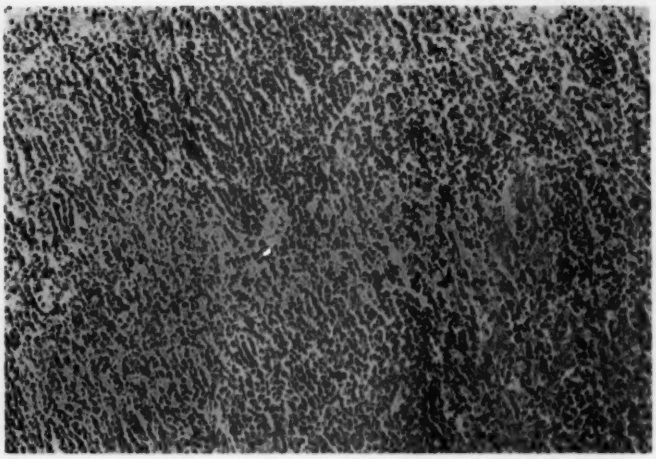

Fig. 6.—Reactive pattern, ammoniacal silver stain. Strong nuclear staining of lymphoid and plasma cells are clearly evident.

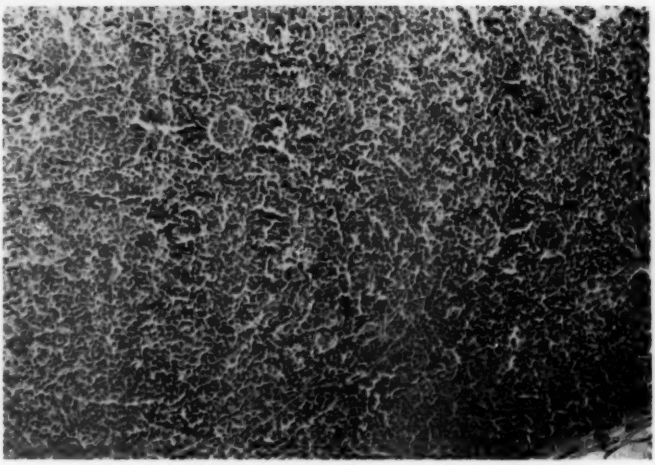

Fig. 7.—Immune pattern, H & E staining. Plasma cells and follicles are somewhat less prominent than in the reactive pattern.

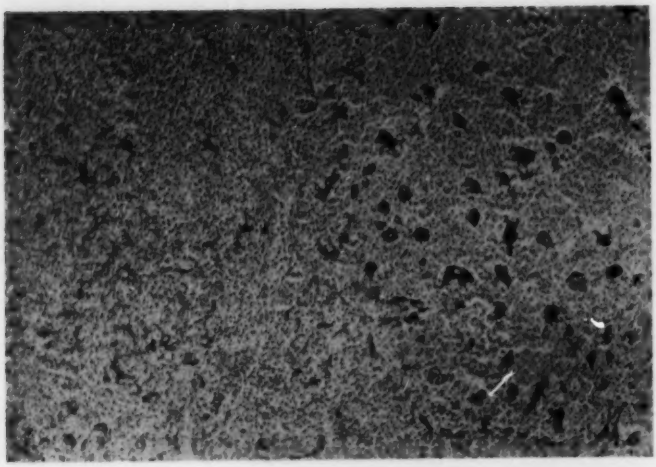

Fig. 8.—Immune pattern, ammoniacal silver stain. Note the hypertrophied "gitter-like" metallophil cells in the follicles. Nuclear staining is absent.

Fig. 9.—Immune pattern, medullary region, ammoniacal silver staining. The intense staining of the sinusoidal and pulp metallophils are well demonstrated.

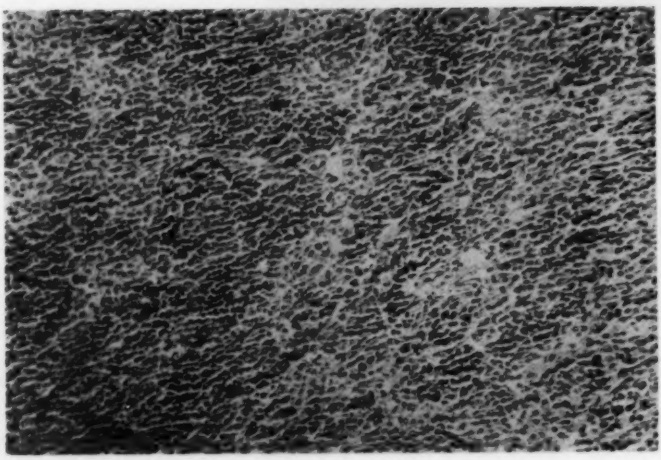

Fig. 10.—Exhausted pattern, H & E stain. Note degenerative changes of sinusoidal and plasma cells in the medulla.

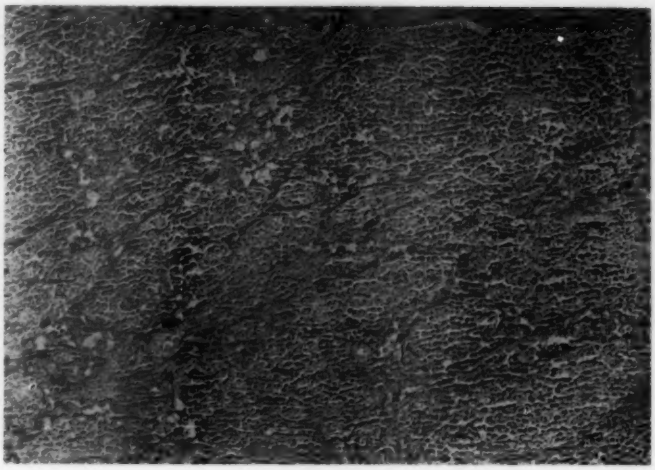

Fig. 11.—Exhausted pattern, ammoniacal silver stain. There is poor staining of all elements. The print was purposely overdeveloped to make it reproducible.

sociated with continued and overwhelming amounts of antigen.

Sinus Histiocytosis (SH).—Such nodes are characterized by distention of the sinusoids by a compact syncytium of somewhat fusiform histiocytes having a distinct eosinophilic cytoplasm. The pulp is largely lymphocytic in type. Follicles and plasma-cell aggregates are minimal. After silver staining, the cytoplasm of the sinus histiocytes stains distinctly. However, the littoral cells of the sinusoids do not stain as prominently as the histiocytes. Thus, the sinus outlines are not distinct. The reticular cells of the pulp are usually less prominent than in the control pattern. Nuclear staining of the lymphoid cells is minimal or absent (Figs. 12 and 13).

Table 1 summarizes the salient features of the various types of lymph node patterns encountered in this study, while Table 2 indicates the relative frequency of the different lymph node patterns as found in the total control and cancer series.

The presence of a spontaneous breast carcinoma in CFW mice was typically associated with reactive changes in the axillary lymph nodes. In the majority of cases, the axillary, brachial, and inguinal lymph nodes had similar patterns, suggestive of a

Fig. 12.—SH pattern, H & E staining. The sinusoids are distended by a syncytial histiocytic proliferation. Follicles and plasma-cell aggregates are minimal.

Fig. 13.—SH pattern, ammoniacal silver stain. Note the distinct staining of cytoplasm of the sinusoidal histiocytes. Nuclear staining of lymphoid cells is minimal and pulp metallophils are not prominent.

TABLE 1.—*Structural Features and Silver Staining of Lymph Nodes from CFW Mice*

	H & E *		Silver Stain *			
					Sinusoids	
Pattern †	Follicle	Plasma Cells	Lymphoid-Cell Nuclei	Pulp Reticular Cell	Littoral Cells	Histiocytes
Control	0-2	0-2	0	2	1-2	1-2
Recognition	0-2	0-2	3-4	0-1	0-1	0-1
Reactive	2-4	2-4	3-4	0-1	0-1	0-1
Immune	3-4	3-4	1	3-4	3-4	2
Exhausted	3-4	3-4	0-1	0-1	0-1	0-1
SH	0-1	0-1	0-1	2	2	3-4

* Graded according to intensity of the feature from 0 to 4.
† See text for details.

response to antigenic stimulation. A distinct nuclear staining was found in 46% of the spontaneous-tumor-bearing mice, in contrast to a value of less than 20% in the series of control mice. Prominent follicles and plasma-cell aggregates were found in 51% of these tumor-bearing cases.

In one-quarter of the spontaneous tumor series the pattern was of the *exhausted* type, whereas this pattern was not found

TABLE 2.—*Relative Frequency of Lymph Node Patterns in Relation to Tumor Size (CFW Mice)*

		% Incidence					
	No.	Control	Recognition	Reactive	Immune	Exhausted	SH
Tumor, cm.²							
1.0	14	21	43	7	—	—	29
1.0-3.9	11	—	18	18	9	27	27
4.0	11	9	—	55	—	36	—
Multiple	7	—	29	14	—	57	—
Metastases *	11	9	18	36	—	44	—
Total	43	9	23	23	2	26	16
Control	27	49	10	—	30	—	—

* Lymph nodes from those mice having lung metastases. Primary tumors varied in size from 1.0 cm² to 4.0 cm.²

in the control mice. Such patterns were frequent in the mice with large and multiple tumors. This pattern was also found in mice with progressively growing implanted tumors. In contrast, the group with small spontaneous tumors was characterized by the absence of the exhausted pattern and the presence of either the *SH* or *recognition* patterns (71%).

Comment

As compared to control mice, the lymph nodes of the spontaneous tumor-bearing mice were characterized by a greater degree of follicular prominence and plasma-cell aggregates. This finding corresponded to the reports of other investigators.[8] It is generally felt that such structural features reflect a response to antigenic stimuli. The observation that 46% of the mice had a nuclear type of silver staining of their lymph nodes is also in keeping with the view that the nodes were being stimulated antigenically.[6]

It would, therefore, seem that the spontaneous tumor was acting as an antigenic stimulus. Albert et al., using isotope techniques, came to the conclusion that implanted and spontaneous breast tumors produced antigenic stimulation of the lymph nodes.[9] However, neither their data nor ours indicated that the *reactive* pattern was associated with any demonstrable anti-tumor-growth effect. In fact, follicular prominence and plasma-cell aggregates were most intense in the lymph nodes of the mice with the large tumors or multiple tumors and in those with lung metastases. The growth of the primary and metastatic tumors, despite reactive lymph nodes, might have several explanations: (*a*) no antibody was produced despite the structural appearance of the lymph nodes; (*b*) the tumor growth potential "overrode" the action of antibody, or (*c*) the antigenic stimulus (and the reactions thereto) was not critically related to the growth of the tumor. The available data do not allow us to make a definitive evaluation of these possibilities.

It is pertinent to note that the *immune* pattern was rare, whereas both the *reactive* or the *exhausted* patterns were found in more than 80% of the group with large tumors. These findings would be consistent with antigen excess associated with progressive tumor growth. However, no conclusions can be drawn as to the relationship between such "antigen" and the growth of the spontaneous breast carcinomas. It is conceivable that the "antigen" concerned might not be critically related to tumor growth. On the other hand, necrosis of implanted mammary carcinoma from mice of the same strain was associated with an *immune* pattern. This would suggest that there is a correlation between necrosis of the implanted tumor and an *immune* pattern in the lymph nodes. It is not known whether the same relationship exists in the case of the spontaneous mammary tumors.

Further data on the relationship between lymph node structure and the growth of the spontaneous breast cancer in mice were obtained by studying the growth of the tumors in nine of the mice for a period of 10 weeks before the mice were killed. In three of these mice, the tumors decreased in size 10% to 53%. The lymph nodes of these three mice had *SH* patterns. The tumors of the other six mice increased 84% to 280%. Their lymph nodes were predominantly of the *reactive* and *exhausted* type. It would seem from these data that a decreased growth rate of cancer tissue was not necessarily associated with the reactive or immune type of lymph node response.

The finding that the *SH* reaction was more frequent in the small-tumor group and that this reaction was found in the three mice having decreased tumor size is suggestive of our previous observations on lymph node structure in human breast carcinoma.[10] Breast cancer patients having *SH* reactions in their axillary lymph nodes were found to have prolonged survivals, even in those cases eventually dying of cancer (Fig. 14). The present observations are consistent with the view that *SH* reactions of the

Fig. 14.—SH pattern, human axillary lymph node from case of breast carcinoma, ammoniacal silver stain. Patient is free of disease eight years postoperatively.

lymph nodes reflect a tumor-antagonistic reaction of the host. The factors resulting in the production of *SH* rather than a *reactive* type of node pattern in individual cancer-bearing animals and patients are unknown.

Since the small-tumor group was also associated with a high incidence of *recognition* patterns and since transitions have been seen between the *recognition* and the *SH* pattern (human data), it appears likely that the *SH* pattern develops from the recognition pattern. In this instance, however, there seems to be a stimulus toward the production of lymphocytes rather than plasma cells.

Summary

Hematoxylin and eosin and ammoniacal silver staining were used to evaluate the microscopic structure of the axillary, brachial, and inguinal lymph nodes of CFW mice bearing spontaneous mammary carcinomas. Distinct lymph node patterns were identified and related to patterns seen after antigenic stimulation. The lymph node patterns of CFW mice bearing spontaneous mammary carcinomas were consistent with antigenic stimulation.

Mice with large tumors or multiple tumors or those with lung metastases had the highest incidence of degenerative changes in their regional lymph nodes (*exhausted* pattern). A similar pattern was found in mice bearing progressively growing implants of spontaneous CFW mammary carcinoma. In contrast, an *immune* pattern was found in the lymph nodes of mice after necrosis of such implanted tumors.

Mice bearing small or partially regressing spontaneous mammary carcinomas were characterized by *recognition* or *SH* lymph node patterns.

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Fat Embolism in Chronic Alcoholism

Control Study on Incidence of Fat Embolism

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The changes found in the central nervous system in association with chronic alcoholism appear to be ill-defined and, apparently, nonspecific. It was at first thought that Wernicke's "superior hemorrhagic polioencephalitis"[1] was specifically linked to chronic alcoholism, and Gudden's atrophy of the corpora mammillaria[2] was somewhat later similarly associated. However, subsequent observations by Neubuerger[3] and others[4] cast much doubt on the specificity of the association between the Wernicke-Gudden changes and alcohol addiction, and it is now accepted that the lesions described by these pioneers are due to concomitant vitamin deficiencies. Indeed, the Wernicke complex is now rarely found in chronic alcoholism, and more recent investigators[5,6] have focused attention on cerebellar atrophy as a prominent finding in habitual alcoholics. Neubuerger,[7] in a recent study of the brains of 42 chronic alcoholic cases, found the most impressive and consistent changes to lie in the cerebellar cortex and to consist of a selective degeneration of the granular layer—only rarely accompanied by serious alteration or loss of the Purkinje cells. In Neubuerger's series[7] the more classical changes were in evidence in varying numbers of cases. Thus, signs of old brain trauma and mild degrees of internal hemorrhagic pachymeningitis were found in isolated cases. Atrophy of the brain was present in less than 50% of cases and was mild and sometimes associated with edema of the leptomeninges. In the group showing atrophy the alterations were rather nonspecific and consisted of thinning of the cerebral cortex, with diminution in the number of neurons. The lesions of Wernicke's syndrome were found in only two cases, while two cases showed some evidence of Marchiafava-Bignami changes in the form of slight focal demyelination of the corpus callosum.

It will be evident, therefore, that, though general dietary improvement has altered the neuropathologic picture of chronic alcoholism, certain diffuse or patchy, ill-defined, and apparently nonspecific changes, such as loss of neurons, focal degenerative lesions, atrophy, and astrocytic proliferation, remain to be explained.[8] Such changes have generally been regarded as nonspecific—similar to those attributable to anoxia, toxins, and vitamin deficiencies.[8] Nevertheless, it is suspect whether any or all of these convenient "causes" suffice to explain the picture in many cases.

Our interest in this problem was aroused by the work of Hartroft and Ridout,[9] who demonstrated that rats fed on choline-deficient diets develop "fat cysts" in their livers and that such large fat globules may rupture into biliary or vascular channels. These workers examined human autopsy material from 30 cases of chronic alcoholism and found "small amounts of embolic fat within the vessels of the lungs and kidneys in a number of cases." After our preliminary findings[10] a more exhaustive study was undertaken, and the results to date are here presented in conjunction with those

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from a consecutive series of routine autopsies.

Material and Methods

Alcoholic Cases.—At autopsy a block 3×2 cm. in area×1 cm. in thickness was taken from each lung—usually from the lateral aspect of the upper portion of the lower lobe or lower part of the upper lobe. Fixation was accomplished in Lillie's buffered neutral formalin,[11] and frozen sections were then cut at 30μ-35μ. These were then stained with oil red O and were counterstained with Mayer's hemalum only when photography was contemplated, as counterstaining renders it difficult to obtain uniformly even sections. The number of emboli per section was counted with use of a × 12 objective and × 10 eyepieces and was recorded in each case together with the areas of the sections. Contiguous intravascular globules of fat were counted as one embolus. Hematoxylin-and-eosin-stained sections of liver were graded 0-4+ as to the degree of fatty infiltration: Grade 1 indicates fat vacuoles in 15% or fewer cells; Grade 2, fat in 15%-30% of cells; Grade 3, fat in 30%-50% of cells; Grade 4, fat in 50% and over of cells. Other than in the alcoholic cases figuring in the consecutive control series, embolism was sought only in those having Grade 2 or greater fatty infiltration of liver. In those cases where the brain was investigated at least two blocks were taken—often 3 or 4—namely, (1) frontoparietal cortex and underlying white matter, (2) basal ganglia, (3) midbrain or pons, (4) white matter around lateral ventricles, including the corpus callosum. Frozen sections of formalin-fixed brain were cut at 25μ for examination and 15μ for photography. Hematoxylin-and-eosin-stained sections of brain (where available) were examined routinely.

Consecutive (Control) Autopsy Series.—In our preliminary investigation,[10] 250 consecutive autopsies were studied, and, in these, fat embolism was found only in cases of trauma, alcoholic fatty liver, and necrotizing pancreatitis. However, in that series only one block each from lung and kidney was examined, and these were not uniformly selected as to size or site. For the present investigation a more thorough study was planned, and a block from each lung was selected and examined as outlined above. Virtually all these blocks measured 3×2 cm., and the area was recorded in each instance.

Alcoholic Psychosis Group.—From the beginning of 1957 to the present time, 51 admissions to our neuropsychiatric unit were in this category. In addition to general measures (extra fluids, usually fruit juices, etc.) these patients received 100 mg. of promazine (Sparine) intravenously immediately upon admission to hospital, followed by the same dose intramuscularly q. 6 h. for the first 24 hours, then sparine, 50 mg. orally q. 4 h., as indicated. They received vitamins in the form of Parlite,* 5 ml. intramuscularly twice daily for the first three days. Fasting morning sputa were collected and examined daily (where possible) as follows: the sputum sample was whisked with an equal volume of saturated solution of oil red O in isopropyl alcohol. After two minutes' standing an amount was placed on a glass slide which was sufficient to form an even layer under a 22×30 mm. cover glass. By measurement this proved to be 0.5±0.1 ml. of the mixture. (The sputum is invariably viscid in these cases.) The number of droplets of fat was then counted in each specimen preparation with use of the magnification already given. All such counts of fat globules in sputa and sections, together with all section-cutting, etc., were performed by the same person, thus affording uniformity. Only definite droplets of fat were counted. Because of the very viscid nature of many sputa it was not always possible to see all droplets present, and so our results tend to err on the low side.

Results

Of the total of 268 autopsy cases there were 200 adults and 68 children (16 years and under). Of the total, 73 (27.2%) showed intravascular fat emboli (Fig. 1*A*).

Trauma Group.—This accounted for 30 cases (41.1%) of all positives and included one child, aged 12 years, i. e., trauma accounted for 29 adult positives (42.6% of all adult positives, or 14.5% of all adult autopsy cases); 93.75% of all trauma cases in the series were positive. The degree of embolization in the trauma group ranged from 1 to 192 emboli per square centimeter of section (mean, 37.3 per square centimeter), but where fat embolism was the apparent or a major contributory cause of death the range was 23 to 192 emboli per square centimeter (mean, 79 per square centimeter)—in the 7 such cases of skeletal trauma in the series.

Soft-Tissue Trauma.—Seven of the twenty-nine (24.1%) adult trauma cases fell in this category, and embolization ranged from

* Parlite (Lederle). Each cc. contains vitamin B_1 2 mg., riboflavin (B_2) 2 mg., *d*-panthothenic acid (sodium salt) 2 mg., nicotinamide 30 mg., vitamin B_6 1 mg., ascorbic acid 100 mg., vitamin B_{12} 5 mcgm.

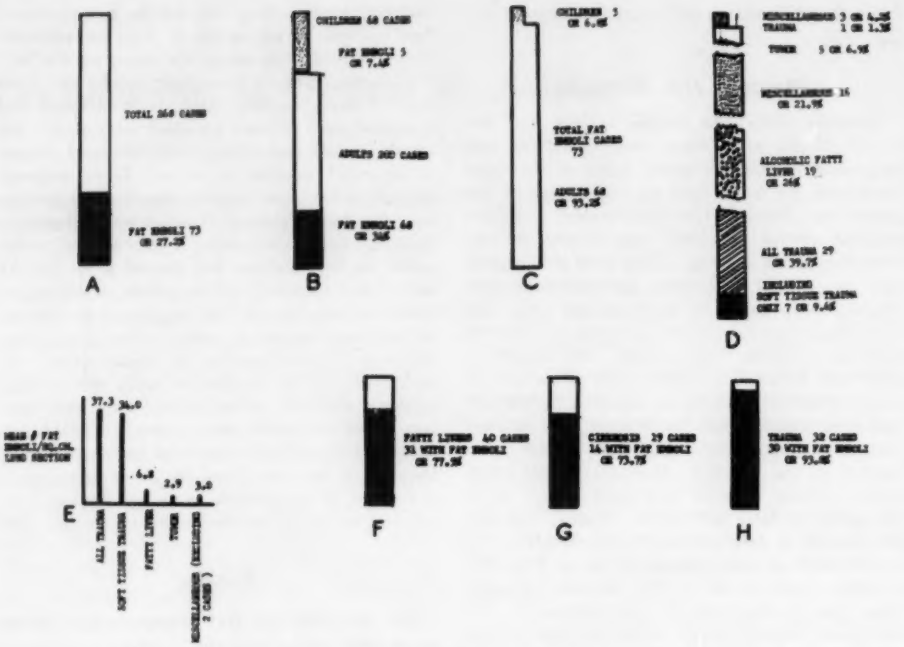

Fig. 1.—Analysis of fat embolism as found in 268 consecutive autopsies.

1.3 to 154 per square centimeter (mean, 34.0 per square centimeter), the lightest being a case of uremia due to electrical burns. The group included two cases of assault and beating, one of which also suffered a cut throat. In the four cases of the soft-tissue-trauma group where fat embolism was assumed to have been a major contributory factor in the fatal outcome, the range of embolism was 9.8 to 154 per square centimeter (mean, 54.3 per square centimeter). The case showing heaviest embolization in

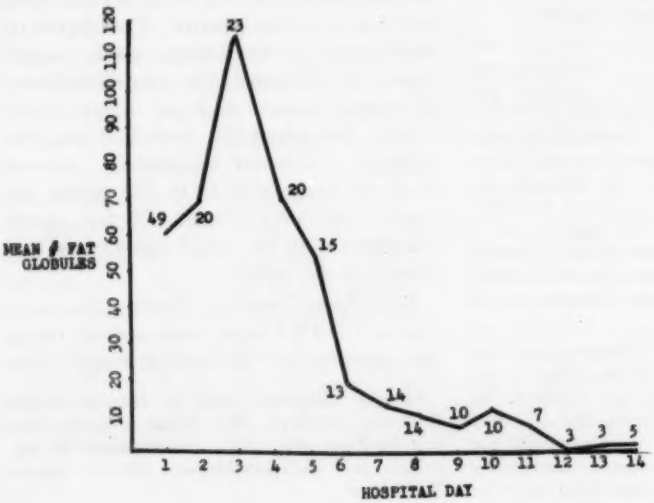

Fig. 2.—Curve of average number of fat globules (10μ-100μ) found on each day of hospitalization in alcoholic psychosis group. The number of cases examined is indicated on the curve for the corresponding day.

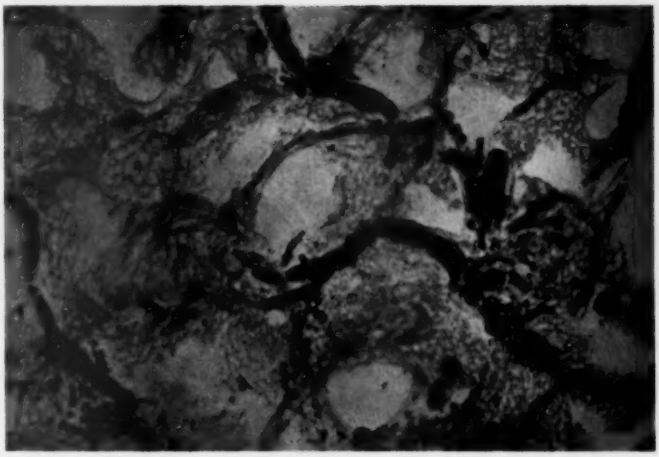

Fig. 3.—Pulmonary fat embolism in a case of soft-tissue trauma. Oil red O with light Mayer hemalum counterstain. Frozen section; reduced 40% from mag. × 100.

this group was a 51-year-old moderately obese woman, who was thrown from an automobile in a road accident. Most of her buttocks had been torn away by the gravel road surface. She died eight days after the accident, never having recovered from her shocked condition and showing a marked respiratory distress and cyanosis (Fig. 3).

Alcoholic Fatty-Liver Group.—Nineteen (9.5%) of the two hundred consecutive adult autopsies fell into this category, and all showed pulmonary fat embolism of some degree (range, 0.5 to 34 emboli per square centimeter; mean, 6.8 per square centimeter). The 19 included 5 cirrhotics, 2 of whom died in hepatic coma (both showing cerebral fat emboli) (Fig. 1 *E*, *F*, and *G*).

Tumor Group.—This was a fortuitous discovery in that in one case suitable blocks of lung without tumor metastases could not be found. Examination revealed occasional fat emboli in endothelized channels which were always closely associated with tumor, the cells of which were markedly fatty. Subsequently a further four cases of this nature were found. In all, in so far as we have been able to determine by careful observation, the fat appeared to be in small vessels devoid of red blood cells and, therefore, presumed to be lymphatics (Fig. 4).

Miscellaneous Group.—In this were placed cases in which there were no obvious or commonly accepted cause of the fat embolism. Nineteen cases were so labeled

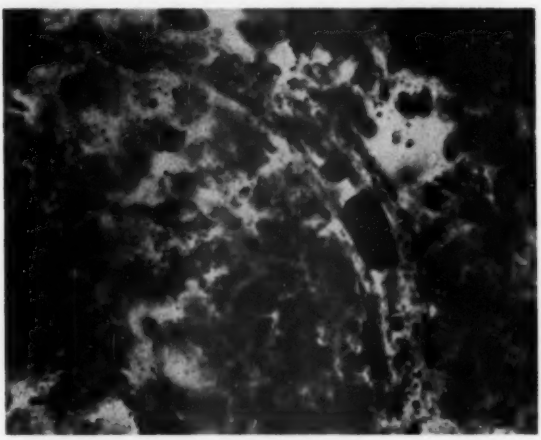

Fig. 4.—Lymphatic fat "embolus" presumed to be derived from neighboring fat-laden tumor-cell metastases in lung; × 300.

(26% of all positives, or 21.9% of adult positive cases; Fig. 1*D*). Only two cases in this category revealed significant embolization, namely diabetic coma in a girl aged 11 years (37 emboli per square centimeter), and secondary amyloidosis in a woman aged 53 years (28 emboli per square centimeter). The degree of embolization in the remainder was slight (range, 1 to 8.6 per square centimeter; mean: 3.0 per square centimeter). In addition to the two cases mentioned, the miscellaneous group included Hodgkin's disease, one; generalized scleroderma, one; idiopathic pulmonary fibrosis, two; atherosclerotic heart failure, two; congenital hydrocephalus, one; Waterhouse-Friderichsen syndrome in an adult, one; ruptured brain abscess in a child aged 22 months; senile ischemic encephalomalacia, one; severe frost bite in an adult, one; acute bronchopneumonia in an old man, one. Five of the group should, perhaps, not be included, since a probable cause of the fat embolism was present in each, namely, carcinoma of pancreas, one; postoperative mesenteric fat necrosis, two; focal necrotizing pancreatitis, two.[12]

Children.—The five positive cases were as follows: lymphatic embolization from pulmonary metastases of a sympathicoblastoma in a 2-year-old girl; ruptured brain abscess in a 22-month-old boy; a 2½-year-old congenital hydrocephalic; a .22 bullet wound of brain in a 12-year-old boy (death within a few minutes of wound infliction); diabetic coma in an 11-year-old girl. With the exception of the last case the degree of embolization was extremely slight in all the children.

Comment

To review the vast literature that has accumulated on the subject of fat embolism since its original description by Zenker,[13] in 1862 (in a crushed railroad worker), is beyond the scope of the present work. Nevertheless, a brief discussion on the general topic appears desirable—if only to place the problem in perspective.

Many authors have, directly or indirectly, minimized the importance of fat embolism and for many reasons, namely, (*a*) because of the frequency with which it is found in routine autopsies[14,15]; (*b*) because of an assumed discrepancy between the amount of embolized fat and the amount of fat available in the traumatized site[16]; (*c*) because of the difficulty in dissociating the symptoms and signs of fat embolism from those of the almost invariably associated shock, as pointed out and clearly discussed by Robb-Smith[17]; (*d*) because of the great diversity of conditions in which it has been claimed to have been found.[16,18-23] For all that, however, the majority of careful investigators clearly recognize the reality and importance of the condition and believe the source of the fat to be local, i. e., the traumatized site.[17,18,24,25]

It is apparent from the literature that much of the confusion might have been avoided had there been a more critical assessment of cases and some attempt at appraisal of the degree of embolization. Thus, it will be apparent from the present investigation that one may get an entirely erroneous and unfavorable impression of the importance of fat embolism by merely stating that it was found to occur in 27.2% of the series. The degree of embolization, which we have here attempted to assess, would appear to be valuable—even with the limitations as to precision inherent in the method. Our own experience has convinced us that careful attention to technique is most important. Thus, as Shields Warren pointed out,[25] many emboli will be lost if sections are cut too thin. Inexperienced workers may confuse inhaled oil droplets, peribronchiolar fat deposits, and pneumonic exudate fat with emboli. Inhaled oil droplets usually derive from the use of oily nasal or oropharyngeal sprays in the elderly; we have found such droplets to display a rather characteristic patchy distribution within groups of alveoli, though some are dragged over alveolar septa during sectioning. Peribronchiolar fat is not uncommon in adults[16]; we have found it in approxi-

mately 20% of those past middle age. Fat deriving from pneumonic exudate is almost always in the form of tiny droplets, intra-alveolar and mostly in phagocytes and leukocytes. It does not appear to be absorbed by lymphatics, and, though we have examined many sections from bronchopneumonic areas, we have found fat embolism in only one such case. It has been said that one may see emboli in sections from one area of lung, while those from other areas are entirely negative.[14,16] Such has never been our experience; indeed, we have been impressed with the uniformity of the findings from different areas of the lungs of any one case —provided such areas were comparable as to presence or absence of disease and degree of respiratory mobility.

Though it has often been questioned whether the amount of fat disturbed at the site of trauma is sufficient to produce the degree of embolization noted, and though it has often been claimed that the fat may derive from such sources as blood lipid, neither of these hypotheses has ever received even the slightest experimental support. Indeed, simple arithmetic tells us that 1 ml. of fluid fat may give rise to no less than 10,000,000 emboli, each measuring $40\mu \times 80\mu$. The fact that fragments of bone and marrow may be found embolized in the lungs of fracture cases [25] is further support for the local origin of the fat. Without particularly seeking this finding, we have found it in one case in the present series (Fig. 5).

Some instances of fat embolization which have in the past seemed inexplicable are now at least open to sound theoretical explanation. For example, in cases of burns [19] it is entirely likely that the emboli derive from traumatized panniculus adiposus, as they probably also do in cases of pure soft-tissue injuries. Likewise, in cases of carbon tetrachloride [20] and phosphorus poisoning [16] it is probable that the emboli arise from the associated fatty infiltration of the liver. It is noteworthy, perhaps, that, to our knowledge, none of the series in the literature give any mention of the number of fatty liver cases in the groups investigated. It is worth recalling also that Scuderi [18] re-

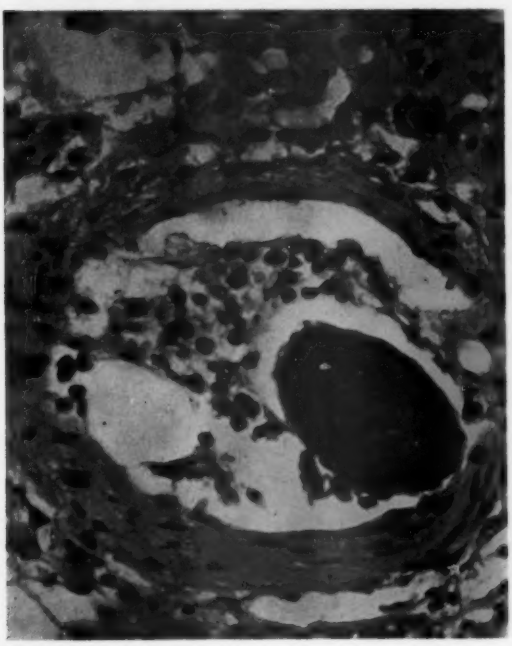

Fig. 5.—Embolized bone and marrow fragment in lung prearteriole from a case of skeletal trauma. Hematoxylin and eosin; × 300.

marked, "It appears as though alcoholics are more predisposed (to fat embolism) than non-alcoholics." While it is possible that still other sources of fat embolism may come to light, one is, nevertheless, tempted to ask how certain we can be that the "miscellaneous group" have not suffered antemortem soft-tissue trauma sufficient to explain the very light degree of embolism usually found in such anomalous cases. Finally, as regards the symptomatology of fat embolism, the work of Whiteley [28] deserves attention. This worker has shown experimentally in rats that the presence of shock greatly increased the symptoms, reduced the threshold dose of injected fat, and led to the arrest of larger emboli in larger vessels. Our own clinicopathologic correlations fully support Whiteley's conclusions.

Chronic Alcoholic Group.—To the time of writing, of 40 cases of alcoholic fatty liver cases investigated, 31 (77.5%) have shown pulmonary fat embolism. In 14 of these cases the brains were examined, and fat embolism was found in 12 (68.5%), while the 2 negative cases showed marked perivascular accumulation of fat-laden scavenger cells—a finding which we have noted in all alcoholics with fatty livers. Of a total of 19 alcoholic cirrhosis cases, 14 (73.7%) have shown fat emboli in the lungs. In nine such cases the brains were available, and fat embolism was found in six (66.6%). It must be stated that in most of the alcoholic group death was attributable to conditions not directly related to alcoholism. However, in certain cases the fat embolism appeared to have played a decisive role in the causation of death, while in others it may well have formed a major contributory factor.

Alcoholic Fat Embolism as Major Factor in Death

CASE 1.—This case has already been described (Reference 27, Case 2).

Sections from the patient's lungs revealed an average of 72 emboli per square centimeter (Fig. 6). Several routine sections of liver showed fused and ruptured fat cysts communicating with the hepatic sinusoids (Fig. 7). Fat emboli were seen in all of four areas of brain sectioned. Acute bronchopneumonia was the immediate cause of death, but it is more than likely that the extremely heavy fat embolization precipitated this.

CASE 2.—Though this 47-year-old traveling salesman had been a member of the A. A. A. for one year, he was still a compulsive though secret drinker. He was found dead in his hotel bed. His roommate related that on the previous evening the salesman had seemed greatly confused, almost irrational, and had complained almost continuously of thirst and a sense of "choking." He had been tremulous and exceedingly restless; he had perspired profusely, and his color was dusky. Finally, he had fallen into a deep snoring gurgling sleep.

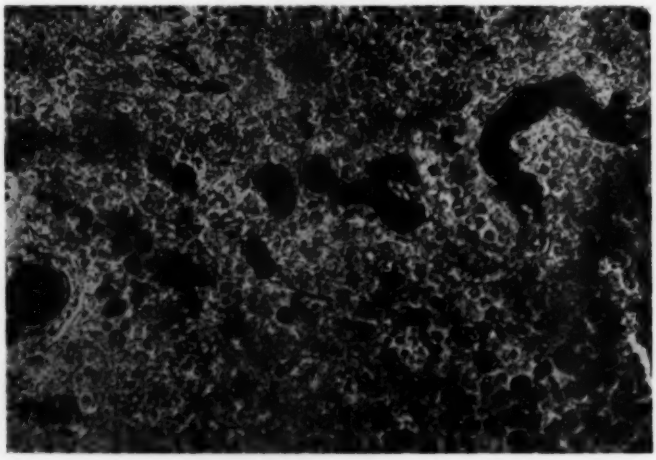

Fig. 6. — Heavy pulmonary fat embolism from alcoholic fatty liver; reduced 40% from mag. × 300.

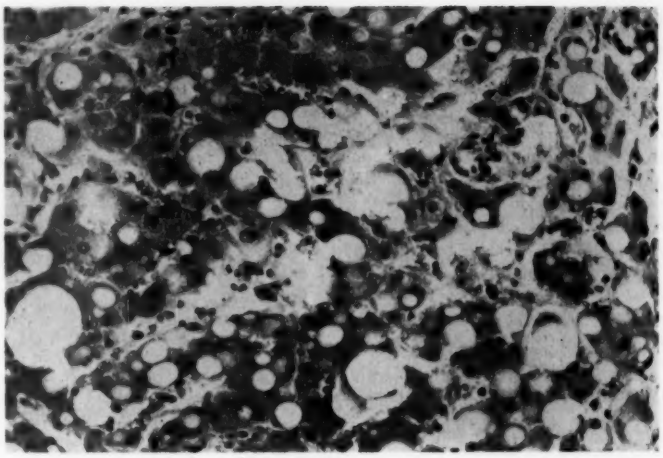

Fig. 7. — Liver from same case as Figure 6. In four areas fat cysts are seen to have fused and, apparently, communicate with the hepatic sinusoids. Hematoxylin and eosin; reduced 40% from mag. × 300.

Autopsy revealed no adequate cause of death—indeed, no possible cause other than fat embolism. This, however, did not appear heavy in the lungs (4.2 emboli per square centimeter) but was disproportionately heavy in the brain. As many as 35 emboli were counted in 1 sq. cm. of a 25μ section taken at the level of the optic tract.

CASE 3.—This chronic alcoholic man, aged 30 years, while in one of his bouts called at a doctor's office at 9 p. m., complaining of shortness of breath and "choking" of two hours' duration. He had

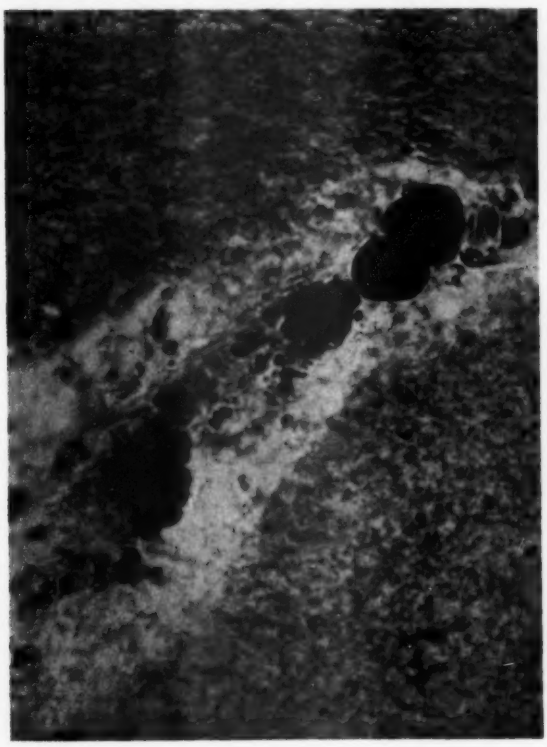

Fig. 8.—Cerebral fat embolism from alcoholic fatty liver. Case 3 of text: subcortex. Frozen section. Fat-laden perivascular scavenger cells are also seen; × 630.

experienced similar attacks toward the end of his alcoholic episodes during the course of the past two years, but none had been as severe as this one. He was adamant that he had imbibed only three bottles of beer that evening. The clinical findings not being very definite, and not knowing the patient's background, the doctor administered morphine, ¼ grain (16.25 mg.), and advised the patient to see his own doctor with a view to seeking consultation on his complaint. His wife subsequently related that he went to bed that night about midnight "in his usual inebriated state." When she retired, at 1 a. m., she noted that he was breathing heavily. When she awoke at 8 a. m. she found that he was dead.

Autopsy revealed Grade 2-3 fatty infiltration of the liver and also fat embolism in the lungs and brain (Fig. 8). Alcoholic concentrations in specimens obtained at autopsy were as follows: blood, 1.62 parts per 1000; urine, 3.66 parts per 1000; stomach contents, 2.36 parts per 1000. It was felt that death was due to respiratory failure occasioned by the combination of acute alcoholism, fat embolism, and morphine.

CASE 4.—This chronic alcoholic woman, aged 62 years, had been drinking for several days, during which she had been showing increasing signs of approaching delirium tremens. She complained of sudden headache, quickly lapsed into coma, and died within two hours of onset of the headache.

At autopsy a large subarachnoid hemorrhage arising from several small "bleeders" within the cortex of the right frontal pole and fatty liver were found. There was well-marked fat embolism in the lungs (34 per square centimeter, Fig. 9) and in the brain. There was no obvious cause for the subarachnoid bleeding, and, though this may have been spontaneous, it was considered probable that fat embolism, possibly along with vitamin deficiency, may have triggered the hemorrhage.

CASE 5.—This 40-year-old man had been a chronic alcoholic. One year prior to his death he had sought medical advice and was for a time placed on disulfiram (Antabuse) prophylaxis. However, on at least three occasions following this he had gone on long drinking bouts lasting several weeks at a time. His latest lapse began one week before his death. On the evening of the night during which he died he was found in a very drunk and very excited state and complained of headache and of "tightness" in his chest. Phenobarbital (phenobarbitone), 1 grain (65 mg.), was given to sedate him, and he was put to bed, where he was found dead on the following morning.

At autopsy there was edema and congestion of the leptomeninges, brain, and lungs; Grade 2 fatty infiltration of the liver, and a blood alcohol level of 3.55 parts per 1000. Aldehydes were present in abundance in the urine (it was later found that he had taken disulfiram [Antabuse] on the day prior to death). Death was presumed to have been due to the combination of acute alcoholism, the effects of disulfiram on alcohol metabolism, fat embolism, and phenobarbital. Fat emboli were abundant in the lungs (22 per square centimeter) and in the brain (Fig. 10).

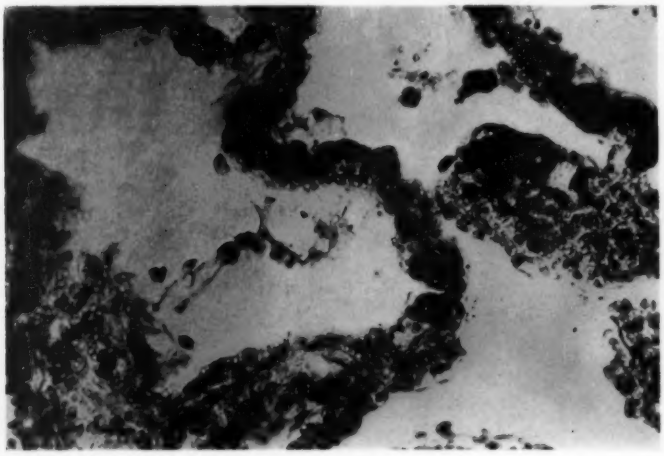

Fig. 9.—Pulmonary fat embolism from alcoholic fatty liver; Case 4 of text. Frozen section; reduced 40% from mag. × 300.

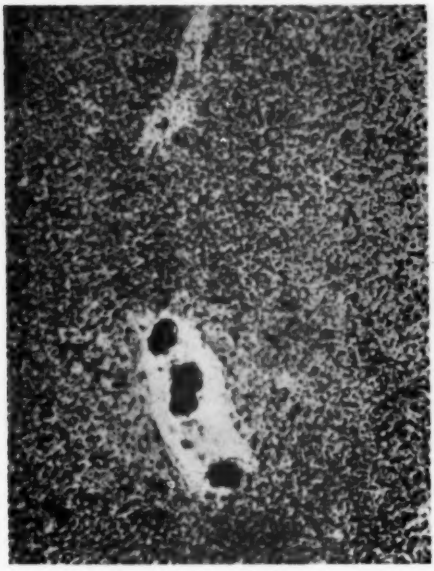

Fig. 10.—Cerebral fat embolism from alcoholic fatty liver; Case 5 of text. Frozen section; × 180.

In addition to the above cases we have experienced seven other instances in which alcoholic-conditioned fat embolism *may* have played a precipitating role in the final illness or drama, e. g., two cases of brain abscess without any of the usual antecedent infections, two cases of suicide where death was instantaneous and where fat embolism (60-70 emboli per square centimeter of lung) *may* have triggered the suicidal impulse; an instance of "hepatic coma" in which biochemical tests and autopsy findings did not reveal a degree of cirrhosis that could be directly incriminated but in which fat studies revealed heavy fat embolization of brain and lung (67 emboli per square centimeter), an instance in which a small dose of barbiturate had apparently precipitated fatal coma but in which moderate fat embolism was found at autopsy in the lungs and brain. The seventh case in which fat embolism contributed materially to the immediately

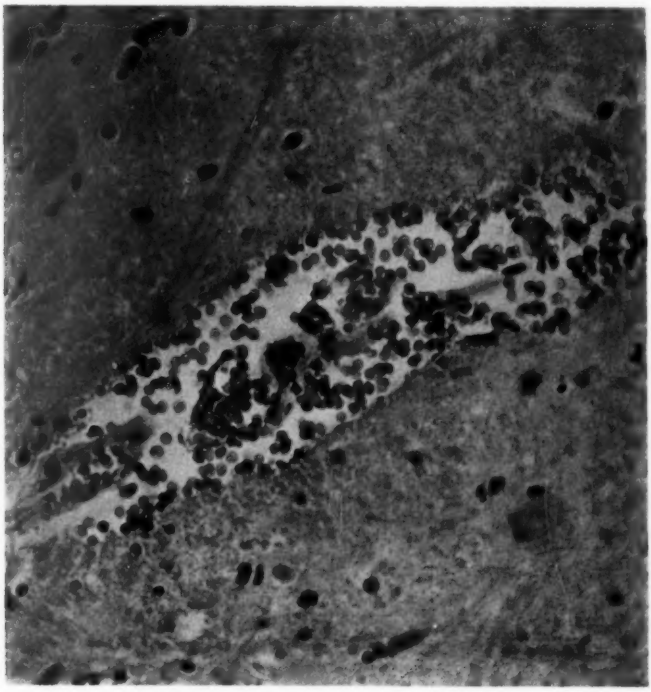

Fig. 11. — Rhexis in Virchow-Robin space in chronic alcoholism. Hematoxylin and eosin; reduced about 5% from mag. × 300.

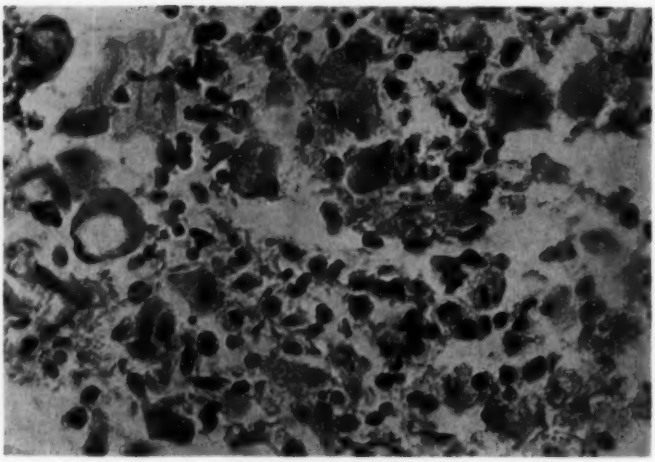

Fig. 12. — Focus of foamy scavenger cells in leptomeninges in case of heavy fat embolism from alcoholic fatty liver; Case 1 of text. Hematoxylin and eosin; reduced 40% from mag. × 630.

indictable fatal process has already been described elsewhere (Reference 27, Case 1).

We do not wish to imply that fat embolism in chronic alcoholism is heavy or consistently present; indeed, on the average, and compared to trauma cases (Fig. 1*E*) it is relatively light—though the group whose average values are shown in Fig. 1*E* contained few cases of heavy embolization. On the other hand, individual cases do show a degree of embolism which is grave by any standard. At the moment we can but guess as to the part played by this embolism in the genesis of the many changes noted in the brains of chronic alcoholics. That some at least of these alterations may be due to the repeated ischemic effect of embolism does not seem unreasonable. Though our search of these brains by conventional methods has been far from exhaustive, we have observed in all pathologic findings which could well be the result of the lodgement of plastic lipid emboli (Figs. 11 and 12), namely, microscopic hemorrhages in the Virchow-Robin spaces and brain substance, focal accumulations of lipid-laden scavenger cells in these spaces and in the leptomeninges, apparent local loss of cells, and microcystic degeneration and glial proliferation of focal and microscopic proportions. No instance of the Wernicke findings was found in any of the brains examined, nor was any other evidence of vitamin deficiency patent. However, it is obvious that more detailed study and experimental work are indicated before definite conclusions may be drawn.

Alcoholic Psychosis Group.—The results of sputum fat-globule assays in the alcoholic psychosis group are of particular interest and further substantiate our findings in autopsy material. It must be mentioned that the group contained all grades of acute brain syndrome attributable to alcohol. Of the 51 hospital admissions in the series, 10 represent two admissions on the part of each of five patients during the course of the study. The sputa of 48 were positive for fat globules; 3 were negative. The ages ranged from 25 to 79 years (mean, 43 years). Eight were women. Of the total admissions in the series, 8 had severe symptoms (full delirium tremens); 15 were moderate in the degree of mental upset; 16 were mild; 9 were very mild, and 3 were young adults (25-34 years) with little more than "hangovers." The sputa in the last-mentioned cases were negative for fat globules. The number of globules of fat found in the specimens was consistently proportionate to the severity of symptoms and signs and appeared also to parallel the duration and severity of the alcoholic history. The ages of the eight severely affected

cases ranged from 30 to 53 (mean, 45 years). The fat globule count in the sputum dropped invariably as the clinical status improved. In three cases, after the initial fall in the count significant increases were noted in the numbers of globules in the sputa. In each of these three cases inquiry revealed considerable deterioration in the patient's condition on the night prior to the morning on which the particular specimen of sputum had been produced. The size of globules appeared to be roughly related to clinical severity—larger globules being found in the more seriously affected patients. Two cirrhotics were included in the series, and both had strongly positive sputa.

Consistent with an embolic basis was the finding of considerable respiratory embarassment in all of the more seriously affected cases. A feeling of "tightness in the chest" or "choking" was commonly volunteered, and cyanosis and effort of respiration were evident in all of the more marked cases. The onset of the mental disturbances usually coincided with the end of a bout of alcoholism, though this was by no means always so. One can only speculate as to the reason for this. Is it a withdrawal phenomenon in the broad sense, or is it related to some protective (? solvent) effect of the alcohol, as has been postulated in the case of ether? [16] It could, of course, equally well be due to simple loading of the liver with fat—a factor which in itself may have something to do with the termination of the craving for alcohol! The elevation in the number of fat globules found in the sputa on the third day of hospitalization is difficult to explain, unless it represents a delay in the appearance in the sputa of the peak embolism which precipitates hospitalization. In our series on the third day 10 cases showed such a peak but 9 cases showed a fall in the number of globules in the sputum.

In the latter half of our study serial blood magnesium levels have been estimated in the alcoholic psychosis group. Our findings to date in this respect support the observation of Flink and his associates [28] that there is a fall in the serum magnesium level in this condition. The work of correlating this with the evidence for fat embolism is continuing.

Summary

Evidence is presented from both autopsy and clinical material that fat embolism is of common occurrence in chronic alcoholics. Fat embolism is advanced as a major factor in the etiology of alcoholic psychoses and may lead to death in some such cases. Repeated fat embolism may be the basis of some, at least, of the changes noted in the brains of chronic alcoholics.

A control study of the incidence and rough quantitation of the degree of fat embolism in 268 consecutive autopsies is also reported.

Paris St.

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Whipple's Disease—Observations on Systemic Involvement

II. Gross and Histologic Observations

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Introduction

In 1907 G. H. Whipple published a paper entitled "A Hitherto Undescribed Disease Characterized Anatomically by Deposits of Fat and Fatty Acids in the Intestinal and Mesenteric Lymphatic Tissues." He proposed the name "intestinal lipodystrophy," believing the disease was due to some unknown disturbance of intestinal function. Other workers have used the term "lipogranulomatosis" and similar descriptive names. In the light of more recent observations suggesting the systemic nature of this disease, and particularly since its pathogenesis is obscure, the eponym "Whipple's disease" seems preferable.

Approximately 70 cases have been reported under the above or related names, a considerable number of which appear to represent other diseases. For instance, Hendrix et al., in their critical review, excluding their own cases, accepted only 14 of the 23 reported cases at that time.

For other views and reviews see papers by Clemmesen,[3] Rosen and Rosen,[4] Plummer et al.,[5,6] Russo,[7] and Puite and Tesluk.[8]

There are no pathognomonic clinical features in Whipple's disease. The diagnosis, at present, can only be established after microscopic examination of suitable material. For over 30 years after Whipple's original report, the recorded cases were diagnosed only at autopsy. Since the reports by Oliver-Pascual and Hendrix et al., an increasing number have been diagnosed during life after abdominal laparotomy and biopsy.

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In most reported instances of this condition, the pathologic findings have been limited to the abdomen. Our five cases have shown a more generalized involvement. The morphologic basis for this concept is the demonstration in many cells of a characteristic particle or inclusion, which has been described in a previous report.[10]

The clinical and roentgenographic features of our first four cases have been reported in detail previously.[8,11] They will be summarized briefly here.

Report of Cases

CASE 1.—A 43-year-old white chrome-plater was first seen at Henry Ford Hospital in September, 1943, with a history of migratory polyarthritis for several months prior to the presenting complaints. The latter included abdominal cramps and diarrhea for three weeks, resulting in an 18 lb. weight loss. The stools were numerous, watery and malodorous; some blood was noted a few days previously. Examination revealed a thin well-developed white man with marked pyorrhea, generalized lymphadenopathy, abdominal tenderness, and a blood pressure of 110/68 mm. Hg. Roentgenograms of the small intestine showed a "typical deficiency pattern." He ran a febrile course and at times appeared to be suffering from intestinal obstruction. An axillary lymph node biopsy and, later, a laporatomy were done to confirm the clinical diagnosis of a retroperitoneal lymphoma. Enlarged mesenteric lymph nodes were found, and one of these was removed. The patient was unresponsive to a course of x-ray therapy and died on the 55th postoperative day following progressive diarrhea and inanition.

The striking gross alterations at necropsy were polyserositis (pleura, pericardium, and peritoneum), with 400 cc. of straw-colored ascitic fluid; enlarged porous lymph nodes, particularly notable in the mesentery, and small bowel mucosal hypertrophy, with a multitude of diffuse minute yellow-

gray plaques. Additional findings included pneumonia, rheumatic heart disease with mitral stenosis, and an infarct of the left kidney.

CASE 2.—A 27-year-old Lithuanian dairy farmer was first seen at the Henry Ford Hospital in October, 1944, complaining of epigastric pain and swollen tender painful joints. His arthritis developed approximately three years prior to admission, and during this time, he had also been subject to variable attacks of "crampy" abdominal pain which had recently become severer. Loss of 27 lb. occurred in the preceding six months.

Examination revealed a thin pallid white man whose skin had a "grayish" appearance. There was lymphadenopathy in the axillary, inguinal, and cervical regions. A vague mass was palpable in the left lower quadrant of the abdomen, and there was some abdominal tenderness.

X-ray studies of the gastrointestinal tract, gallbladder, and vertebral column were normal. Chest x-ray showed a slight thickening of the interlobar fissure on the right side. During his hospitalization, there was an afternoon fever each day, with episodes of abdominal pain, chills, and diarrhea.

Laparotomy revealed a thickening of the wall of the small bowel, thickening and induration of the mesentery, and many large mesenteric lymph nodes, one of which was removed. He was given x-ray therapy to the abdomen and supportive therapy and was discharged. Weight loss, diarrhea, and pigmentation of the skin were progressive, and he died at another hospital two months later.

Autopsy disclosed numerous hemorrhagic nodules in the skin of the legs, intestines, and kidneys. The mesenteric lymph nodes were markedly enlarged, tan-orange, and porous.*

CASE 3.—A 35-year-old Lithuanian factory worker (a brother of Case 2) was first admitted to the Henry Ford Hospital in 1951 because of painful, hot, swollen, and tender joints of two-and-one-half years' duration. His second admission was in August, 1953, with the chief complaints of abdominal pain, chills, fever, and weight loss.

Examination revealed a chronically ill emaciated white man with a general hyperpigmentation of the skin. His blood pressure was 110/68 mm. Hg. Generalized lymphadenopathy was most prominent in the axillary and inguinal regions, and a poorly defined tender mass was palpable in the left epigastrium. X-ray studies showed a "deficiency pattern" of the small intestine and findings compatible with Marie-Strümpell arthritis in the sacroiliac joints. Chest films revealed enlargement of the right azygous and bilateral peritracheal lymph nodes. Since a working diagnosis of Hodgkin's disease was considered, an axillary and an inguinal lymph node were removed for biopsy. While in the hospital, the patient ran an intermittent undulating febrile course, with chills and night sweats. Shortly after the lymph node biopsy, a laparotomy was performed, and a large rather firm nodular tumor mass was found in the leaves of the mesentery, extending into the retroperitoneal space. Similar tissue was found along the lesser curvature of the stomach. He was treated with cortisone, corticotropin (ACTH), and other supportive therapy. Soon after he was discharged from the hospital his symptoms recurred and gradually increased in severity. Terminally he developed a right lower lobar pneumonia, with abscess formation, and he died on March 7, 1954.

Gross autopsy findings consisted of serositis involving the pericardium, peritoneum, and right pleura, with 400 cc. serosanguineous fluid in the peritoneal cavity and 200 cc. of straw-colored fluid in the right hemithorax.

The mesenteric and peripheral lymph nodes were enlarged, with a yellow porous cut surface. The boggy small intestine showed a velvety buff mucosa in most areas, while in others it showed diffusely distributed minute gray-yellow "grains" on a reddish background. There were also a right lower lobe lobar pneumonia and an esophageal ulcer.

CASE 4.—A 49-year-old white married factory worker was first seen at the Henry Ford Hospital in December, 1953, with the chief complaints of intermittent diarrhea, upper abdominal distress, and intermittent migratory polyarthritis. In 1950 an exploratory laparotomy had been performed and the appendix, removed. Enlarged mesenteric lymph nodes were interpreted on gross examination as mesenteric lymphadenitis. In 1953, a cholecystectomy was performed at another hospital.

Physical examination showed him to be thin and pallid, with tanning of the skin of the neck, face, forearms, and hands. His blood pressure was 90/50 mm. Hg. Palpation revealed abdominal fullness and resistance and inguinal and right axillary lymphadenopathy. X-ray studies revealed a typical "deficiency pattern" of the small intestine and a Marie-Strümpell arthritis. The chest films were normal. On the basis of an axillary lymph biopsy, a diagnosis of Whipple's disease was made. The patient was treated symptomatically for intermittent attacks of anemia, diarrhea, and polyarthritis and was last heard from in January, 1957, three years after the diagnosis was established.

CASE 5.—A 59-year-old white man had been seen over a period of 13 years, with intermittent fever, polyarthritis, anemia, diarrhea, weight loss, and generalized lymphadenopathy. The blood pressure was 130/80 mm. Hg. X-ray examination disclosed gallstones and poor motor function of the small

* Necropsy data and microscopic slides were obtained from Dr. G. W. Ramsey, Washington Hospital, Washington, Pa.

intestine. Mesenteric lymph node and jejunal biopsy performed at the time of cholecystectomy and cholecystogastrostomy showed the changes of Whipple's disease. Hydrocortisone therapy produced temporary subjective and objective improvement, but he died in a state of shock on Dec. 10, 1955.

Necropsy disclosed polyserositis, with approximately 250 cc. of straw-colored fluid in the peritoneal and each pleural cavity. The mesentery was thickened by enlarged, tan lymph nodes having numerous and small porous areas giving the cut surface a honeycombed appearance. The mucous membrane of the small bowel appeared hypertrophic, containing diffusely dispersed minute gray-yellow plaques. Enlarged axillary and inguinal lymph nodes did not show the striking porosity of the mesentery nodes. Other findings were pulmonary edema with infarction and a patent cholecystogastrostomy.

Histologic Study

I. Methods

Alternate sections of paraffin-embedded formalin-fixed tissue were stained with hematoxylin and eosin and subjected to the periodic acid-Schiff (P. A. S.) technique, with and without diastase digestion. Fat stains were performed on frozen sections of formalin-fixed tissue. These and the other special stains performed are those in use in most tissue laboratories and can be found in the book by Lillie.

II. Findings

The morphologic basis for defining the systemic involvement in this disease is a characteristic cytoplasmic particle found most commonly in histiocytes. This sickle-form particle (S. P.) is markedly Schiff-positive and is diastase-resistant. In our routine hematoxylin-and-eosin-stained sections of formalin-fixed tissue the cytoplasm of these cells appears as a lacy eosinophilic network about irregular gray-blue areas. The cells containing these particles have been called sickleform-particle-containing (S. P. C.) cells. The material examined from each patient is listed in the Table.

Heart: The heart was involved in all cases examined. Foci of S. P. C. cells were found within the substance of the valves, in the subendocardium, and within the interstitial connective tissue of the myocardium. Only a few of these foci were associated with

*Distribution of Lesions in Whipple's Disease**

Case	1	2	3	4	5
Heart	+	○	+	○	+
Lung	+	○	+	○	+
Spleen	+	○	+	○	+
Liver	+	+	+	○	+
Gallbladder	+	|	|	○	+
Pancreas	+	○	+	○	+
Kidney	+	○	|	○	+
U. Bladder	|	○	|	○	|
Prostate	|	○	|	○	|
Testis	|	○	|	○	|
S. Vesicle	|	○	|	○	|
Vascular	|	○	○	○	+
Thyroid	|	○	|	○	○
Adrenal	+	○	+	○	+
Parathyroid	○	○	○	○	○
Esoph.	+	○	+	○	+
Stomach	+	+	+	○	+
Duodenum	+	+	+	○	+
Jejunum	+	+	+	○	+
Ileum	+	+	+	○	+
Appendix	|	○	+	○	○
Rt. Colon	+	○	+	○	+
Lt. Colon	+	○	+	○	+
Mesenteric L. N.	+	○	+	+	+
Periph. L. N.	+	+	+	+	+
Serosa	+	○	+	○	+
Skin	○	|	|	○	○
Brain	+	○	+	○	+
Bone	+	○	+	○	○
Pineal	○	○	○	○	○
Chorioid Plexus	○	○	○	○	○
Thymus	○	○	○	○	○

* + indicates involvement with S. P. C. cells; —, insignificant or questionably significant involvement with S. P. C. cells; 0, tissue not available.

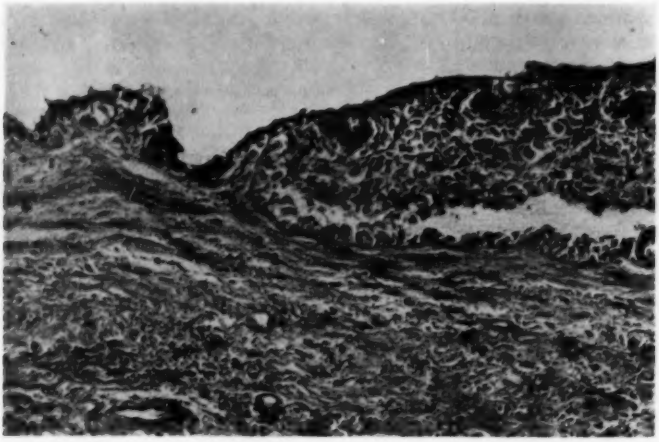


Fig. 1 (Case 1).—Nodular elevation of visceral pleura as a result of focal accumulations of S. P. C. cells. Hematoxylin and eosin; reduced 10% from mag. × 115.

other inflammatory changes. The least extensive involvement was found in Case 1, where Aschoff bodies and other stigmata of rheumatic heart disease were present. The positive P. A. S.-reacting material of the "basophilic degeneration" of myocardial fibers and lipochrome pigments, as well as other non-diastase-resistant material, was noted.

Lungs: Although the lung showed involvement in all cases, this was usually less extensive than that of the heart. Groups of S. P. C. cells were found primarily in the interalveolar septa and interstitial connective tissue. An occasional free macrophage in the alveoli showed the characteristic cytoplasmic particles. There was no correlation between the type of degree of inflammatory process in the lung and the number of S. P. C. cells or the number of particles per cell. In a few instances, these cells were found with portions of the cytoplasm projecting into the alveoli from the septa, resembling the well-known leukocytic emigration through the walls of capillary vessels.

The serosa and subserous connective tissue showed focal accumulations of these cells, simulating granulomas and resulting in nodular elevations of the visceral pleura (Fig. 1). No necrosis or giant cells were seen.

Spleen: The capsule contained S. P. C. cells in all cases examined. Cases 3 and 5 showed some of these cells in and around practically every Malpighian corpuscle. Plasma cells containing Russell bodies were not numerous but when present in the P. A. S.-stained sections showed a positive reaction. The shape, size, and intensity of the reaction differed from that of the sickleform particles.

In Case 5, in which there was marked phagocytosis of hemosiderin, some of the macrophages contained the characteristic particles in addition to hemosiderin. However, the majority of the S. P. C. cells were filled with particles and occurred in different sites from those containing the iron pigment (Fig. 2). In Case 1, there were fewer S. P. C. cells in the Malpighian corpuscles, but large numbers of them were related in the areas of infarction.

Liver: Sickleform particles were found in Kupffer cells and in histiocytes of the capsule and portal areas. In the latter site, aggregates of S. P. C. cells measuring up to 0.8 mm. in diameter were not uncommon (Fig. 3). Occasionally these cells appeared definitely related to blood vessels. In other areas there was a peritubular infiltration primarily about the biliary radicles. Digestion with diastase before applying the P. A. S. method to sections of the liver removed most of the positively reacting material of the glycogen-like compounds and facilitated the demonstration of the charac-

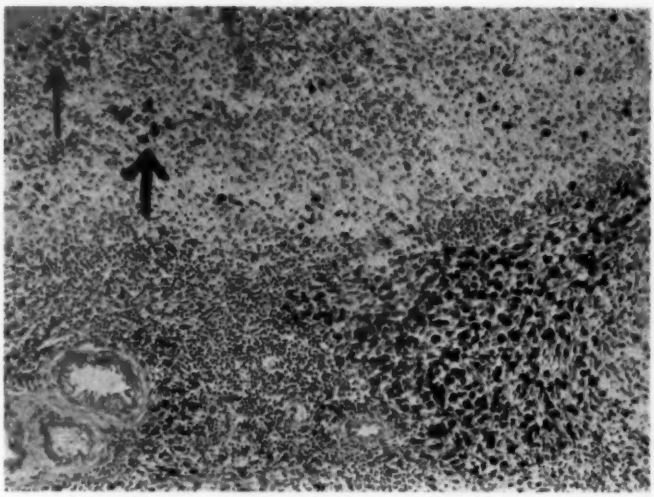


Fig. 2 (Case 5).—Spleen; arrows indicate two small foci of hemosiderin-containing macrophages; remainder of cells with dark cytoplasm, including the focus of cells in lower right quadrant, are S. P. C. cells. Periodic acid-Schiff; reduced 10% from mag. × 50.

teristic particles in the cells. There was a minimal fibrotic reaction associated with the portal aggregates.

Pancreas: All cases examined showed varying degrees of interstitial infiltration by S. P. C. cells. It was least in Case 5 and greatest in Case 1. In the latter case, there was diffuse involvement about the acini, while the insular tissue was usually spared.

Gastrointestinal Tract: S. P. C. cells were found throughout the gastrointestinal tract. The most marked involvement was in the

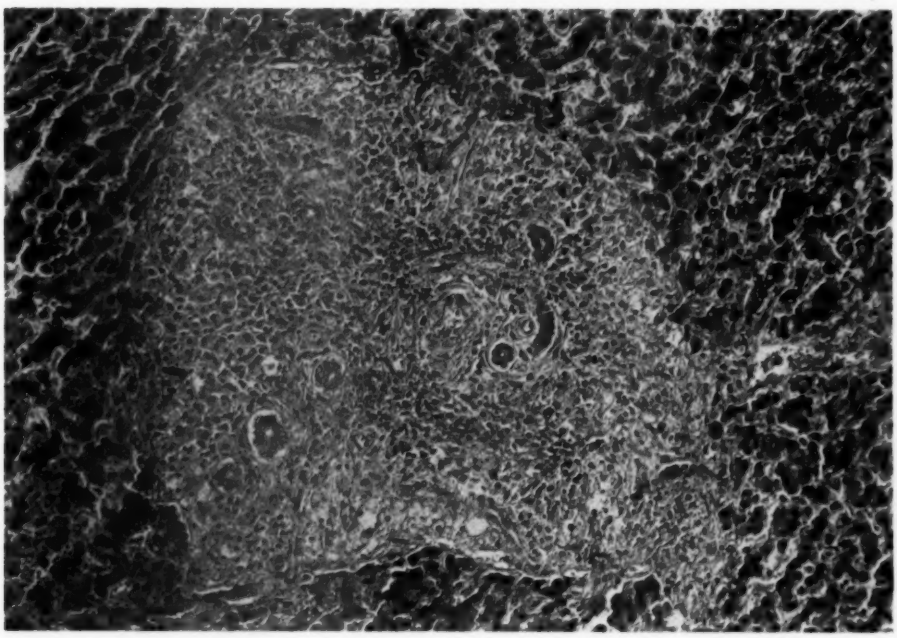


Fig. 3 (Case 1).—Aggregate of S. P. C. cells in liver. Hematoxylin and eosin; reduced 15% from mag. × 115.

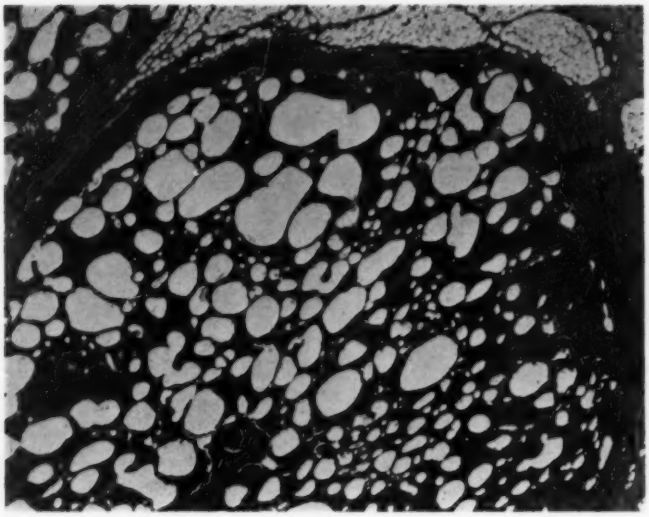


Fig. 4 (Case 3).—Porous mesenteric lymph node from specimen in Fig. 10. Note similarity to Figure 6. Hematoxylin and eosin; reduced approximately 15% from mag. × 25.

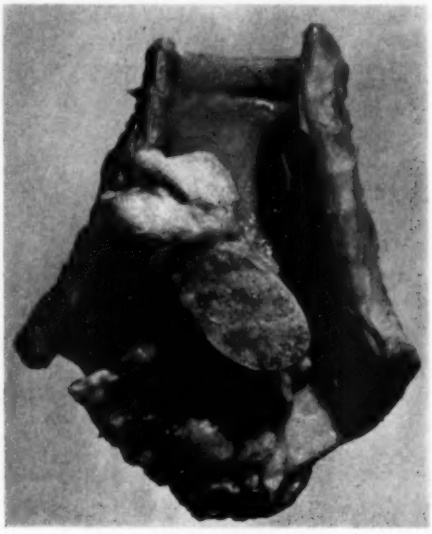
small intestine, where the mucosa was primarily involved, the villi often having the classical "clubbed appearance." The characteristic cells were found in all layers, as well as in the mesentery. There was little or no evidence of active inflammatory reaction associated with these cells.

The esophagus constantly showed infiltration of the outer muscular layer and the paraesophageal connective tissue, but this was less marked than in other parts of the alimentary tract. In Case 2 these cells were demonstrated in all layers associated with superficial ulcers. The stomach and colon showed a similar type of involvement as the esophagus, but in addition the characteristic cells were found in the mucosa and within the lymphoid follicles. The vermiform appendix in Case 3 had large numbers of these cells aggregated in the lymphoid tissue.

Mesentery and Retroperitoneal Soft Tissues: These tissues were diffusely involved, particularly intensely in the periadrenal and perirenal tissues. However, very few of these cells were found in the periureteral and perivesical tissues. The S. P. C. cells in these areas had less tendency to be overstuffed with particles, generally having one-third to one-half the complement seen in the mucosa of the small bowel.

Urogenital System: The ureters, urinary bladder, prostate, seminal vesicles, and testis showed no significant involvement. No aggregates were found, even in areas of inflammation. The kidney showed minimal interstitial involvement in Case 3. Case 1 showed a moderate number of these cells in an area of infarction.

Fig. 5 (Case 3).—Porous tracheobronchial lymph node near carina. Compare with Figure 10.



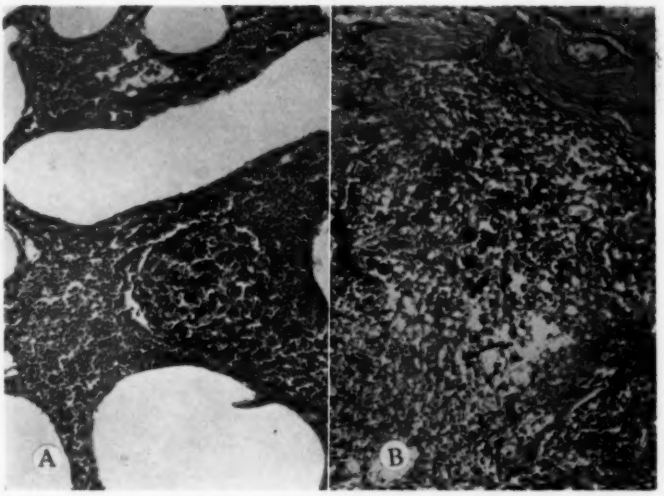


Fig. 6.—*A*, Case 2, mesenteric lymph node. Note darkly staining cytoplasm of S. P. C. cells in germinal center. Periodic acid-Schiff; reduced approximately 25% from mag. × 170. *B*, Case 4, right axillary lymph node of S. P. C. cells in subcapsular zone.

Lymph Nodes: Grossly and histologically it is easy to distinguish two types of nodal involvement, the porous and the nonporous types. The porous nodes (honeycombed nodes, chyladenectasis) are most commonly observed in the mesentery (Fig. 4) but have been described in other sites (Fig. 5). In both types of nodes the characteristic particles are found primarily in the nonlipid-containing histiocytes (Figs. 6). Usually the cells lining the sinuses were the first involved, but it was rare not to see groups of S. P. C. cells in the follicles, centrally or peripherally.

In the porous lymph nodes, we have not observed these cells within the "eosinophilic coagulum" which is often present at the site of future pores or cystic spaces. Lymph nodes from every major anatomical group that we have examined showed definite involvement.

Endocrine Glands: The adrenal gland was the only endocrine tissue showing significant involvement. Aggregates of S. P. C. cells were found in both cortex and medulla. Some were associated with foci of necrosis and inflammation, while others were not.

Central Nervous System: Involvement of the brain was noted in both cases where material was available. The S. P. C. cells were present in large aggregates in focal areas (Fig. 7), and in smaller collections throughout the rest of the brain. The sections of the meninges studied showed no significant involvement.

Other Tissues: Involvement of other mesothelial surfaces was similar to that described for the pleura. A few larger blood vessels showed occasional small subendothelial aggregates of S. P. C. cells and individual endothelial lining cells of many smaller vessels contained the characteristic particles. The bone marrow had focal involvement; the cytoplasmic particles were found in cells lining the sinusoids and within the centers of lymphoid follicles. The skin and subcutaneous tissues showed no definite infiltration, while the skeletal muscles had few cells in widely scattered areas.

Comment

The clinical features of Whipple's disease are not diagnostic. It has occurred most often in middle-aged men, whose chief complaints are of vague abdominal pain, weakness, diarrhea, and weight loss. In many cases these sprue-like symptoms are preceded by a migratory or recurrent polyarthritis. Evidences of dietary deficiencies, hypotension, and polyserositis are often

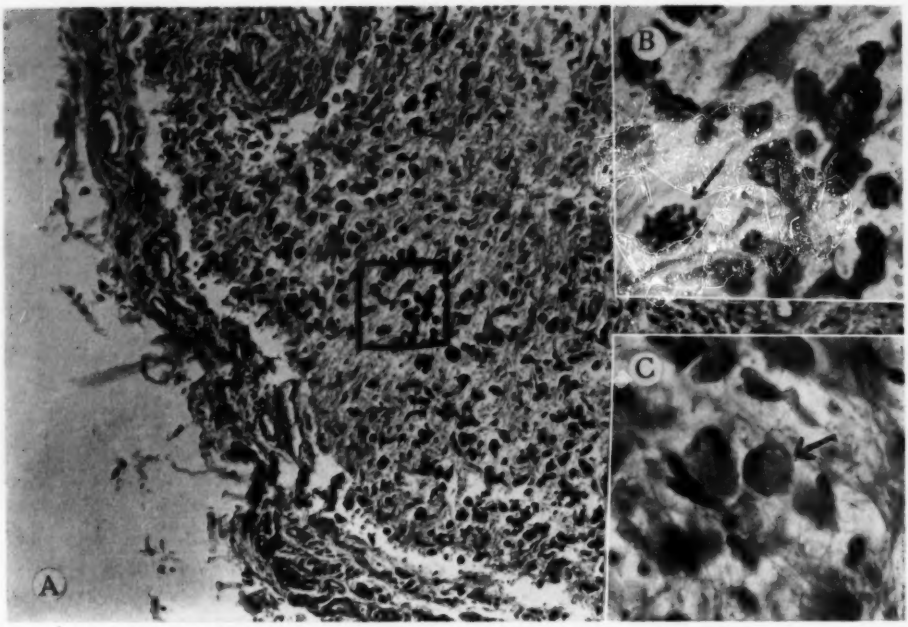


Fig. 7 (Case 3).—*A*, hypothalamic region near pituitary stalk, showing infiltration by S. P. C. cells. Periodic acid-Schiff; reduced approximately 15% from mag. × 115. *B*, insert showing detail from marked area of *A*. Arrow points to S. P. C. cell. Periodic acid-Schiff; reduced approximately 15% from mag. × 720. *C*, arrow points to S. P. C. cell from same area as *A*. Note negative image of sickleform particle. Hematoxylin and eosin; reduced approximately 15% from mag. × 720.

present. About one-half of the reported cases have enlarged peripheral lymph nodes. Less commonly observed are fever and epithelial pigmentation similar to that of Addison's disease.

Roentgenographically the small intestine shows a typical "deficiency pattern," while extraintestinal manifestations may include enlargement of mediastinal and retroperitoneal lymph nodes, widening of the duodenal loop, and changes in the sacroiliac joints like those of Marie-Strumpell's disease.

The laboratory findings are generally those compatible with the malabsorption syndrome. More frequent findings are a microcytic hypochromic anemia, an elevated fecal fat content, reduction in serum protein, a flat vitamin A tolerance test, a delayed oral glucose tolerance test, and an elevated erythrocyte sedimentation rate. Less often, leukocytosis or eosinophilia and reduction of serum calcium and potassium are observed.

The pathologic manifestations of this disease can be divided into two general groups, the lipid and the nonlipid changes. The lipid changes are manifest both extracellularly and intracellularly. The extracellular deposits of fat and fatty acids have been stressed traditionally and need no further emphasis here.

The recognizable intracellular lipid changes are cytoplasmic and are generally in cells capable of phagocytosis. The lipid-containing histiocytes are most prominent in the mesenteric and other porous-appearing lymph nodes, where a type of lipogranulomatosis is observed. The significance of this latter change is unknown. To deepen the mystery, one need only reflect that among the innumerable histiocytes in the intestine and all other sites, only a rare "foam" cell or giant sudanophilic cell is demonstrable. At present, the exact role of the lipid-containing histiocytes in this disease is also unknown.

The role of the lipid-filled histiocytes (foam cell, xanthomatosis cell, foamy macrophage, sudanophilic macrophage) in the intestine has been misinterpreted in the past and may account in part for the lack of descriptions of involvement of other tissues. The prominent lipid accumulations in the mesenteric lymph nodes no doubt influenced the interpretation of the interglandular macrophages or histiocytes in the intestine. In addition, the terms foam cell, or foamy cell and foamy macrophages, have been used interchangeably without reference to their actual fat content or sudanophilia. A study of Figures 3, 4, 7, and 8 of the original case report [1] illustrates this point.

For 40 years after the report by Whipple,[1] the fatty changes were considered the major pathologic alteration, and during this same period, the diagnosis had never been established before death. This classical or traditional concept was summarized in 1947 by Rosen & Rosen [4]: "It is our opinion that the typical lesions of Whipple's Disease as described by him were the massive accumulations of intracellular and extracellular fat in the small intestine, and its draining lymph nodes with dilatation (probably resultant) of mesenteric lymphatics. . . ." During this "lipid" era, there were occasional observations of extra-abdominal involvement, primarily in lymph nodes, in which lipid-containing histiocytes (foam cells) and/or "chyladenectasis" were described. Figure 5, of a tracheobronchial lymph node from our Case 5, is an example of such an extra-abdominal "chyladenectasis."

Unfortunately, many earlier workers stressed the importance of *intracellular* deposits of lipid in the small intestine. However, Whipple [1] observed that most of the fatty material was located *extracellularly* within the bowel wall and predominantly in the lacteals. In his Figures 3 and 4 the vast majority of the interglandular stromal cells, in the mucosa, do *not* contain fatty vacuoles, nor were they stained in the Marchi preparation (his Figure 2).

The importance of the nonsudanophilic macrophage was reemphasized by Black-Shaffer,[13] who also reported that the nonsudanophilic material in the macrophages of the intestinal mucosa and the mesenteric lymph nodes was probably a glycoprotein because it reacted positively with leukofuchsin after periodic acid hydrolysis.[14] The sudanophobic or "P. A. S.-positive macrophage" has been the subject of many histochemical studies,[15-19] which suggest that the nonlipid cytoplasmic material is in a mucopolysaccharide-protein complex. However, there is no complete agreement on this point, or on other staining properties or reactions of this material.

Although the concept of the "P. A. S.-positive macrophages" was of considerable help in confirming the diagnosis of Whipple's disease, most authors to date have not reported involvement beyond the confines of the small intestine and the mesenteric lymph nodes. Occasional reference was made to the proximal colon,[13] in addition to the extra-abdominal lymph node involvement noted above. In 1952, Upton [16] reported observing "mucin-containing foam cells" at sites of inflammation in the endocardium, the pericapsular adipose tissue of the adrenal glands, and the stroma of the portal trinities of the liver. Fisher [19] also noted in cervical lymph nodes cells similar to those described by Upton. However, because of the less intense positive reaction of these cells with the periodic acid-Schiff stain, their similarity to cells in other pathologic states, and the nonspecific character of the morphologic picture in both Upton's case and his own, he questioned their significance and stated, "biopsies of peripheral lymph nodes cannot be utilized to establish the diagnosis of this disease."

The recognition of the S. P. C. cell with its characteristic sickleform particle has aided our study of the systemic involvement in this disease.

The characteristic particle occurs in a wide variety of lipid- and non-lipid-containing cells, commonly nonepithelial and capable of phagocytosis. Until more spe-

cific information is available, we have used a descriptive name for this particle and the cell which contains it. The problems related to the use of the word "macrophage" for these cells, namely, foamy macrophages or sudanophobic macrophages, have been mentioned above; this pertains also to the combined term of P. A. S.-positive macrophage. In addition, these particles are not only "P. A. S.-positive" but are also readily demonstrated by a number of special stains, e. g., mucihematein stain, the Grocott and Gridley fungus stains, the Gram stain, and the Warthin Starry silver technique. In hematoxylin-and-eosin stained sections of formalin-fixed tissue in our laboratory, the sickleform particles appear as gray-blue material separated by lacy pink cytoplasmic strands. Fixation in different solutions can markedly alter many staining reactions of the S. P. C. cells, including complete loss of affinity for hematoxylin. In some tissues, the sickleform particles can be seen clearly as negative images, and this was often evident in the non-lipid-containing histiocytes seen in sections stained with Sudan IV. An example of the phenomenon can also be seen in the giant cell depicted by Whipple[1] (his Fig. 4) near the top center of the photograph.

The S. P. C. cells were found in a wide variety of tissues (Table). The quantitative distribution varied in different sections of the same case, but this was generally less than the difference observed between cases. The failure to demonstrate significant involvement in certain sites in some cases may be due to (1) actual distribution of the lesions (2) size of the samples studied (approximately 1200 slides were examined in this study) or (3) pathologic state of the tissue. The relationship of recognizable inflammatory changes to the localization of these cells is not clear and appears to have no constant effect.

The urogenital system, gallbladder, skin, and all endocrine glands except the adrenal seemed resistant to significant involvement. The bone marrow and blood vessels showed minimal localized involvement. Every section of lymph node examined showed significant numbers of S. P. C. cells. These came from practically all the major anatomical groups, and they ranged in size from 1.3 mm. to over 5 cm. The central nervous system localization was both focal and diffuse.

In addition to our five cases, we were also able to study histologic sections from the cases reported by Upton[16] and Fisher.[19] † S. P. C. cells were found in all slides studied, which included the small intestine, liver, and mesenteric and peripheral lymph nodes.

Of particular interest was the case reported by Fisher.[19] The patient was a 27-year-old housewife, and at the time of his report she was alive and well, approximately three and one-half years after the diagnosis had been made after laparotomy and biopsy of a mesenteric lymph node. The cervical lymph node, removed six weeks before laparotomy, also showed significant involvement with S. P. C. cells. Therefore, the peripheral lymph node involvement occurred at least 42 months before death.

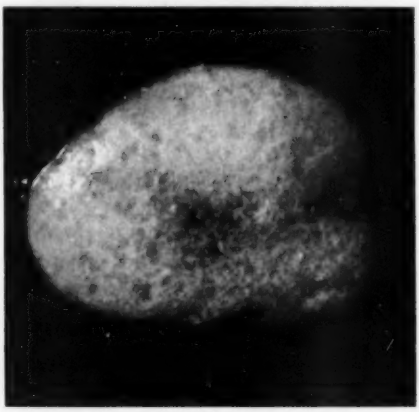


Fig. 8 (Case 3).—Mesenteric lymph node removed after laparotomy approximately seven months before death. Note most cysts filled with an oily substance; reduced approximately 45% from mag. ×4.

Gross changes are not nearly so widespread as are the microscopic changes, us-

† The material from these cases was obtained from Dr. A. J. French, Ann Arbor, Mich., and Dr. J. B. Hazard, Cleveland.

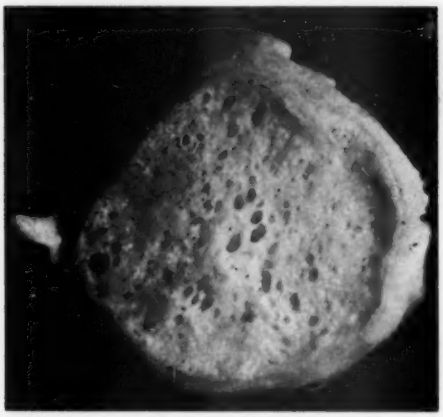


Fig. 9 (Case 3).—Mesenteric lymph node removed after necropsy. Note empty cysts as a result of manipulation. This lymph node was taken from area indicated by an arrow on Figure 10; reduced approximately 45% from mag. × 4.

ually being prominent only in the intestine and lymph nodes. The mesenteric lymph nodes characteristically were enlarged, and the cut surface had a porous or a spongy appearance (Figs. 8, 9, and 10). The cysts or pores in general do not exceed 2 mm. in diameter and contain an oily or fatty substance, or on occasion a white powdery chalk-like material, or were empty. Extra-abdominal lymph nodes occasionally resembled these but were usually not porous. The nonporous nodes, in addition to an increase in size, occasionally showed small (1 mm. or less) yellowish-white nodules.

The external surface of the mesentery was smooth and shining, while the cut surface showed pale yellow fat surrounding the cystic mesenteric nodes. The small intestine generally appeared boggy, and its mucosal surface was a feathery, creamy white. If autolysis was present, minute yellowish-white flecks or grains (about 0.5 mm. or less) could be seen on the mucosal surface against a reddish background (Fig. 11). No fat deposits were visible grossly.

The polyserositis was nonspecific, varying from fibrinous to fibrous. In our autopsied cases, no gross evidence of thoracic duct obstruction was demonstrated. This has been the general experience, although retrograde injection techniques have not been used to study the patency (or lack of it) of thoracic duct. Another deficiency in the pathologic study of Whipple's disease is the paucity of data regarding the joint changes in view of the striking arthritis seen clinically, which usually precedes the intestinal symptoms.

The etiology and pathogenesis of Whipple's disease are unknown. The observations recorded here add little to our knowledge of the fatty changes in this disease. The widespread systemic involvement,

Fig. 10 (Case 3).—Cut surface of mesentery, showing numerous porous lymph nodes. Note similarity to Figure 5.

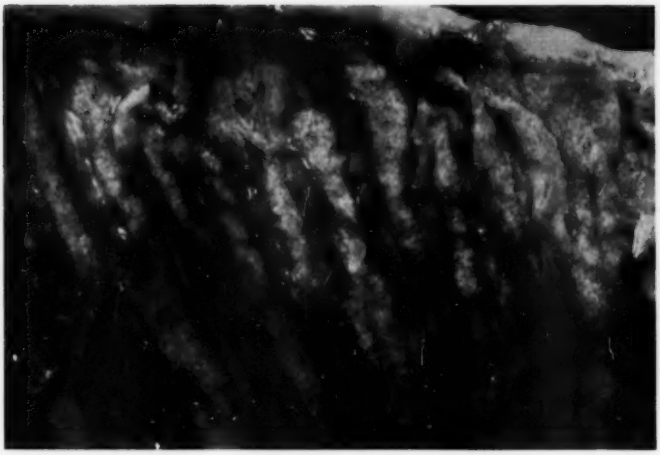


Fig. 11 (Case 3).—Small intestine with characteristic feathery creamy white mucosa (upper) which is less conspicuous in lower half of photograph due to autolysis and manipulation; reduced approximately 40% from mag. × 4.

and especially that of the central nervous system, implicates the blood stream in the transmission of some pathogenic agent or factor. A storage-disease-like distribution is apparent in the necropsy findings. Involvement of the peripheral lymph nodes, which may be involved at least 42 months before death, has allowed diagnosis to be made after biopsy of such nodes.

Summary

Pathologic observations on five cases of Whipple's disease are presented. There was systemic involvement by characteristic cells containing sickleform particles (S. P. C. cells). The relationship of these S. P. C. cells to some of the pathologic changes in this disease is discussed.

The photographs were made by Mr. Arthur Bowden.

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In Vivo Production of a Ceroid-like Pigment in Chickens Given Gossypol

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Gossypol is a yellow pigment with polyphenolic characteristics which is obtained from the gossypol glands of cottonseed. A discussion of the chemistry of this pigment is given by Bailey.[1] The complexity of the structure of gossypol is shown by the variety of reactions which the compound undergoes.[1] Many studies have been made referable to the physiologic and pathologic effects produced by gossypol.[2-5] Recently we have observed a ceroid-like pigment in the intestines, liver, and spleen of chickens fed gossypol in the form of pigment glands.[6] In the present experiment observations have been made on the occurrence of a ceroid-like pigment in chickens after the intravenous and intramuscular injections and the oral administration of gossypol.

Methods and Materials

Forty-two day-old New Hampshire chickens were fed a synthetic type diet for an 18-day period. The diet contained 62.6% glucose monohydrate, 25% Drackett C-1 assay protein, 3% soybean oil, 1.36% vitamin mixture, and 8.03% mineral mixture. The vitamin mixture supplied the following quantities per kilogram of diet: 4.0 mg. thiamine hydrochloride, 6.0 mg. riboflavin, 15 mg. calcium pantothenate, 100 mg. nicotinic acid (niacin), 12 mg. vitamin B_{12}, 6.0 mg. pyridoxine hydrochloride, 2.0 mg. folic acid, 0.5 mg. menadione, 2,000 mg. choline chloride, 33 mg. penicillin, 20 mg. *p*-aminobenzoic acid, 1,000 mg. inositol, 0.2 mg. biotin, 10,000 I. U. vitamin A, 3800 I. U. activated 7-dehydrocholesterol (vitamin D_3), 7.5 gm. methionine, and 4 gm. aminoacetic acid (glycine). The salts mixture contained the following quantities per kilogram of diet: 14.5 gm. $CaCO_3$ 42.1 gm. $CaHPO_4 \cdot 2H_2O$; 9.5 gm. NaCl, 1.1 gm. $MnSO_4 \cdot 5H_2O$, 5.7 gm. $MgSO_4 \cdot 7H_2O$, 1.6 gm. $FeC_6H_5O_7 \cdot 5H_2O$, 16 mg. $CuSO_4$, 14 mg. $ZnCl_2$, 40 mg. KI, 0.5 $CoCl_2 \cdot 6H_2O$, and 5.72 gm. KCl. Gossypol, in the form of pigment glands, was added at the level of 0.4% of the diet.

The birds were placed on a standard commercial ration when the gossypol-containing food was discontinued on the 18th day. On the 19th experimental day, 11 of the chicks were killed; on the 26th, 10; on the 33d, 10, and on the 43d experimental day, 11. Histologic sections were made from the liver and stained with hematoxylin and eosin. Many of the specimens were stained for lipid by the oil red O technique and for hemosiderin by the Prussian blue reaction.

To study the effect of intravenously injected gossypol, a 1.0% solution of gossypol was suspended in isotonic saline to which was added 5.0 ml. of a 1:10 dilution of polysorbate 80 (Tween 80). This preparation was kept in the refrigerator at 7 C for a period of five months. One milliliter was injected intravenously into the leg vein of 13 chickens 40 days of age. Five chickens of corresponding age and size were given 1.0 ml. of only the polysorbate 80 in saline. Two chickens were given an intravenous injection of the gossypol, and 24 hours later the dose was repeated. Two chickens that received an intravenous injection of gossypol died within 25 minutes. The remaining six gossypol-injected chickens and the controls were killed 2 to 20 days later. Histologic studies were made from the liver and spleen. These were stained with hematoxylin and eosin.

In the third experiment gossypol was injected locally into the pectoral muscle. The same gossypol-polysorbate 80-saline preparation was used as was given intravenously, and it was prepared a short time before it was injected. The pH of this prepa-

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ration was 3.62; the pH of the polysorbate 80-saline preparation was 3.84. Injections were made intramuscularly on alternate days into the pectoral region of 20 chickens 4 weeks of age. One milliliter was injected on the first; 0.5 ml., on the second; 1.0 ml., on the eighth, and 1.0 ml., on the ninth experimental day. Five of the chickens were killed 72 hours after the last injection of the gossypol; five, 10 days after, five, 17 days after, and five, 35 days after. Histologic sections were prepared from the pectoral muscle, the liver, and the spleen. These were stained routinely with hematoxylin and eosin. Select tissues were stained by the Perl and the periodic acid-Schiff techniques.

A single 1.0 ml. intramuscular injection of the gossypol-polysorbate 80 preparation five months after being prepared was given to five chickens 40 days of age averaging 490 gm. in weight. One milliliter of the polysorbate 80-saline solution was injected into the left pectoral muscle. Two of these chickens were killed after 5 days and three after 14 days. Histologic studies were made of the pectoral muscle. They were stained with hematoxylin and eosin. Select specimens were stained with osmic acid.

Gossypol was added to cottonseed oil (Wesson Oil) (20 mg. per 1.0 ml.) for intramuscular injection. One milliliter of this preparation was injected into the pectoral muscle on the right sides of six chickens. One milliliter of the corn oil was injected intramuscularly on the left sides of the same birds. Four of the chickens were killed after 24 hours and two after 48 hours. Five chickens of similar age and size were given intramuscular injections of 1.0 ml. of cottonseed oil containing a 1.0% concentration of Black E dye. Two of these were killed after 24 hours; one, after 48 hours, and two, after 72 hours. Sections were prepared from the pectoral muscle and stained with hematoxylin and eosin. Select specimens were stained with osmic acid for fat.

Experimental Results

In the first experiment an attempt was made to determine the length of time the ceroid-like pigment remained in the liver after the injection of the gossypol. The liver from 10 of the chickens fed gossypol and killed on the 19th day, 24 hours after the gossypol-containing food was discontinued, was compared with the liver of 8 chickens killed 24 days after the gossypol was discontinued. Of the first group of 10 chickens, 9 had a 4+ concentration of gossypol pigment in the liver, as indicated by the oil red O stain; 1 chicken had a 3+ concentration. Of the eight chickens killed 24 days later, one had a 3+; one, a 2+; three, a 1+; two, a trace, and one did not show any gossypol pigment in the liver.

The liver and spleen of the chickens killed on the 19th, 26th, 33d, and 43d experimental day were stained with hematoxylin and eosin and they showed a progressive decrease in the amount of gossypol pigment. Essentially no pigment was present in the liver and spleen of the 11 chickens killed on the 24th day after the intake of gossypol in the food was discontinued.

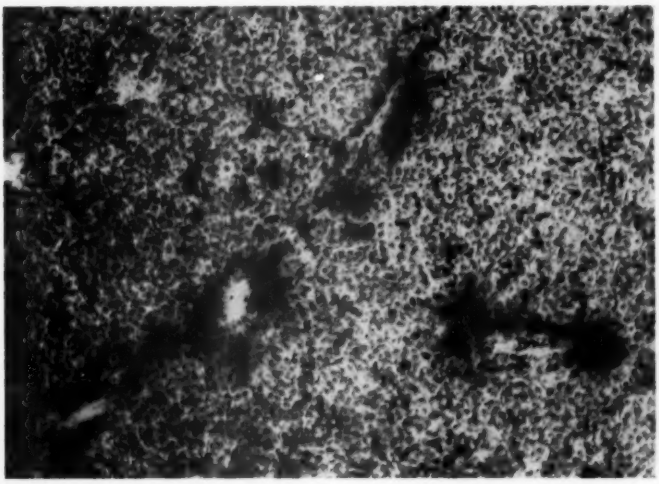

Fig. 1.—Oil red O-staining material is distributed through the hepatic lobule. It is concentrated, however, about the portal triads. Oil red O; reduced 10% from mag. × 170.

The distribution of the pigment in the liver of these chickens, as shown by the oil red O preparation, is interesting. The globules of fat vary in size from 1 to 6μ. They are widely distributed throughout the liver but concentrated around the blood vessels in the portal areas (Fig. 1). There seems to be an absence of lipid-staining material around the central veins. Globules of oil red O-staining material are present in the lumen of some of the hepatic blood vessels. Some of the globules seem to be attached to the endothelial cells of these blood vessels; others appear to be either free within the sinusoids of the liver or in the Kupffer cells. Globules are present in the hepatic cells.

Some of the bile ducts within the liver have a concentration of the oil red O-staining material about their periphery. An occasional epithelial cell lining a bile duct has a globule of red-staining material either in or on the surface of the cell.

It is of interest to observe that all the pigments in the oil red O preparation do not give a positive reaction for fat. Some of the pigment remains yellowish-brown in color. Collections of pigment are present in which only some of the granules stained red. The pigment in the liver of the gossypol-fed chickens usually does not stain positive for hemosiderin; however, few small granules of blue-staining material are present in the sinusoids and in the Kupffer cells associated with the granules of yellowish-brown pigment.

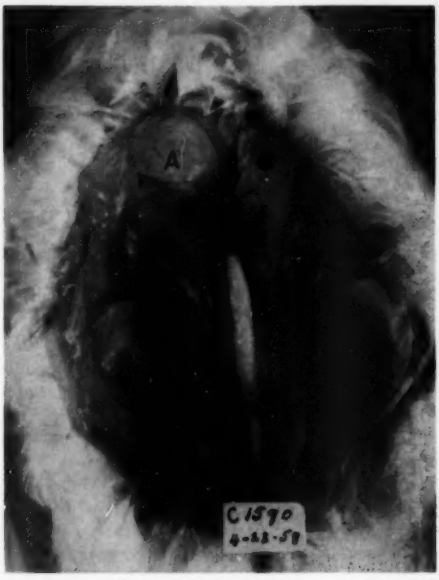

Fig. 3.—Five days after 1.0 ml. of gossypol in polysorbate 80 was injected intramuscularly (*A*). There is a large area of necrosis. A similar amount of polysorbate 80 was injected into the opposite pectoral muscle. There is no macroscopic necrosis (*B*).

Fig. 2.—Twenty-four hours after the intramuscular injection of 1.0 ml. of gossypol in corn oil (10 mg. per 1.0 ml.). Necrosis is present (*B*). No necrosis is present in the opposite pectoral muscle given an injection of an equal volume of corn oil (*A*).

In this study the chickens given the intravenous injections of gossypol have very little pigment in the liver and spleen. Two of the birds died 15 and 25 minutes after the intravenous injection of the gossypol.

Extensive necrosis occurred in the pectoral muscle after the local injection of gossypol in both cottonseed oil and polysorbate 80 (Figs. 2 and 3). Twenty-four hours after the intramuscular injection of 20 mg. of gossypol in cottonseed oil there was necrosis of the muscle, with an accompanying inflammatory process (Fig. 2). The muscle after 48 hours had macrophages filled with yellowish-brown pigment. Acute inflammation, with a minimal amount of necrosis and many polymorphonuclear leukocytes,

Fig. 4.—The muscle shows extensive necrosis in the area injected with gossypol in polysorbate 80. Hematoxylin and eosin; reduced 10% from mag. × 380.

occurred where cottonseed oil and Black E dye were injected intramuscularly in each of five chickens given 1.0 ml. and killed 24 to 72 hours later. No yellowish-brown pigment, however, was present.

Necrosis occurred in the muscle where gossypol in polysorbate 80 was injected (Fig. 3). The necrosis and the inflammatory reaction were similar to that occurring where gossypol in cottonseed oil was injected. Degeneration of the muscle occurred in the areas given injections with only polysorbate 80 in saline; however, no yellowish-brown pigment was present.

Five of the twenty chickens, given injections on four different occasions with gossypol in polysorbate 80 and killed 72 hours after the last injection, showed local areas of necrosis accompanied by macrophages filled with yellowish-brown pigment

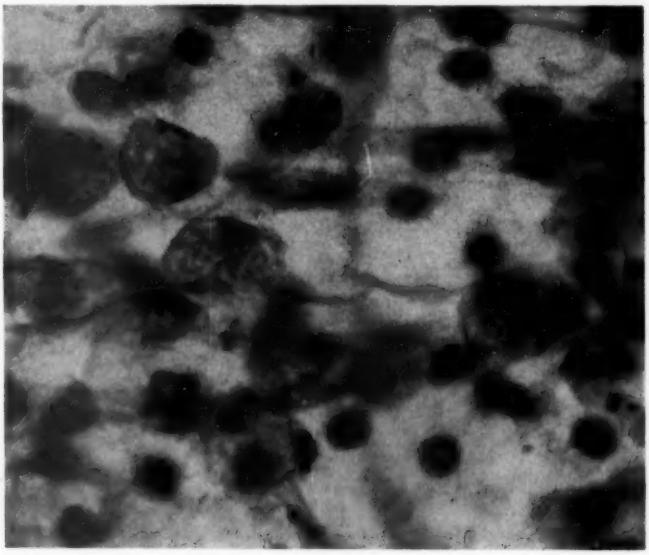

Fig. 5.—Macrophages filled with yellowish brown granules of pigment. Hematoxylin and eosin; × 1083.

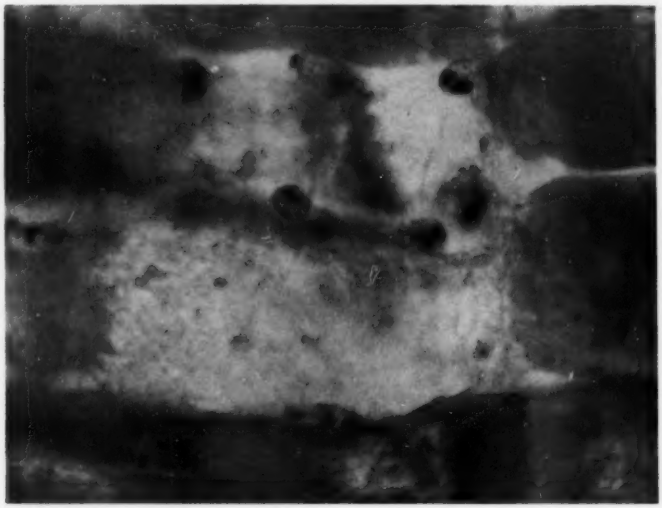

Fig. 6.—Focal area of necrosis and macrophages within the muscle fibers. Hematoxylin and eosin; reduced 10% from mag. × 1083.

(Figs. 4 and 5). A small amount of the pigment was found locally in the chickens killed on the 10th and 17th days after the last intramuscular injection; however, no pigment was found in the five chickens killed 35 days after the last injection of the gossypol.

The acute degenerative changes occurring in the pectoral muscle after the local injection of gossypol are shown in Figure 6. The muscle at the site of injection shows coagulation necrosis. Muscle fibers less severely injured show focal areas of necrosis and vacuolation within the sarcolemma. Some of these injured muscle fibers stain positive for fat with the osmic acid stain (Figs. 7 and 8) and red when stained by the periodic acid-Schiff technique (Fig. 9).

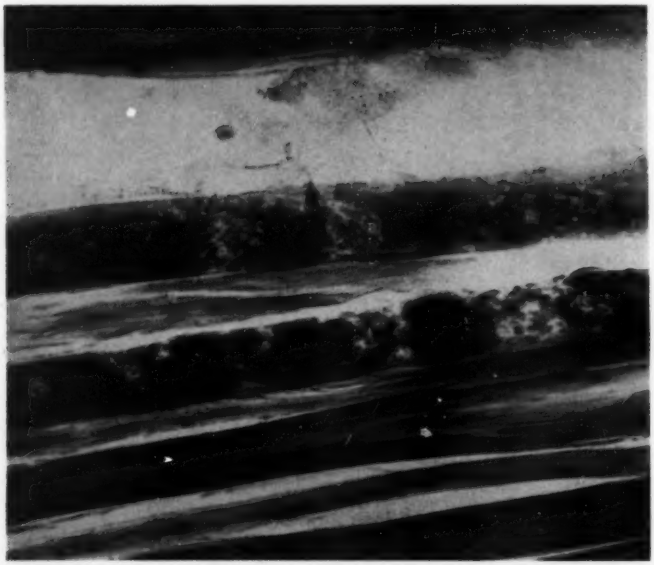

Fig. 7.—The individual muscle fibers show focal areas of necrosis 72 hours after the local injection of 1.0 ml. gossypol in corn oil. These fibers stain black with osmic acid. Osmic acid; × 300.

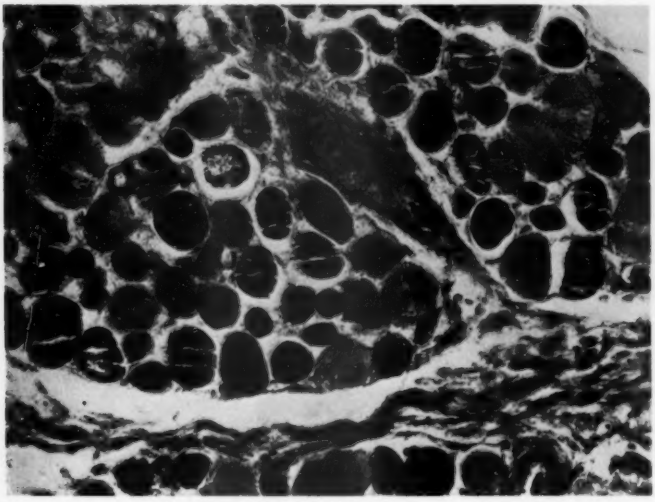

Fig. 8.—Many of the individual muscle fibers injured by gossypol stain black with osmic acid. Osmic acid; reduced approximately 10% from mag. × 300.

Forty-eight to seventy-two hours after the local injection of gossypol many macrophages are present, with granules of yellowish-brown pigment within their cytoplasm (Fig. 5). Sometimes these macrophages are present within the sarcolemma of the degenerating muscle (Fig. 10). The inflammatory reaction within the muscle regresses, and ultimately a scar forms with few, if any, macrophages present with pigment.

The pigment in the liver and spleen after the ingestion of gossypol and in the pectoral muscle after the local injection of gossypol are similar. The pigment is yellowish-brown and granular in the hematoxylin and eosin stain (Fig. 5), periodic acid-Schiff-positive (Fig. 9), oil red O- and osmic acid-positive, and negative for hemosiderin. Dr. Samuel Thompson,[7] of the Army Nutritional Laboratory, examined this pigment and made some additional interesting histochemical observations. 1. The pigment exhibits a light greenish fluorescence in unstained deparaffined sections mounted in glycerin (glycerol) when examined with

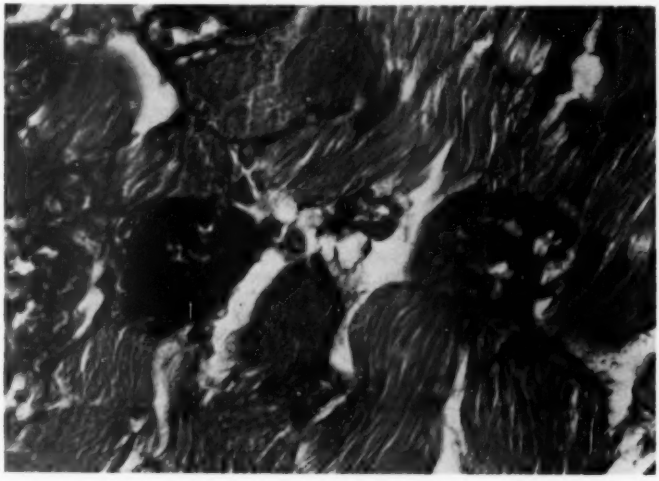

Fig. 9.—Individual muscle fibers, injured by intramuscular injection of 1.0 ml. gossypol 72 hours previously, give a positive periodic acid-Schiff reaction. Periodic acid-Schiff stain; reduced 10% from mag. × 665.

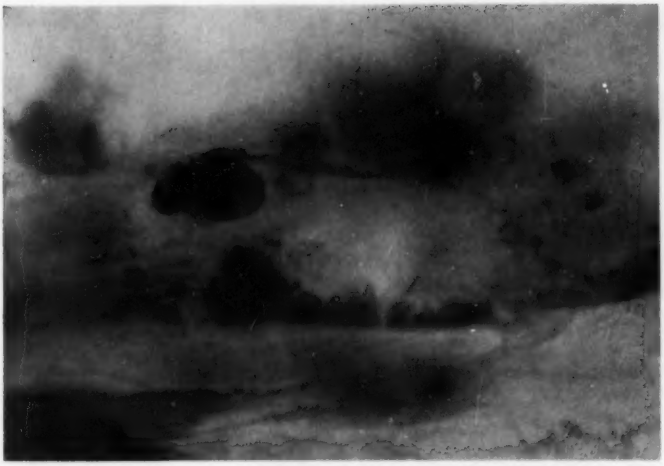

Fig. 10. — Macrophages in the sacrolemma of a degenerating muscle fiber. These cells are filled with the yellowish-brown pigment. Hematoxylin and eosin; reduced 10% from mag. × 1425.

ultraviolet light. 2. The pigment does not either react with or reduce osmium tetroxide. 3. The pigment does not dissolve in the fat solvent when the sections are subjected to hot methyl chloroform by refluxing in a Soxhlet condenser for eight hours. 4. The pigment is not bleached with hydrogen peroxide. 5. The periodic acid-Schiff affinity of the pigment is blocked by acetylation. 6. The pigment as observed under polarized light is not birefringent.

Comment

A yellowish-brown pigment occurs locally in chickens when gossypol in the form of pigment glands is injected intramuscularly. This pigment is similar to that previously reported in the mucosa of the intestine and in the liver and the spleen of chickens fed gossypol.[6,7] The histologic observation, as made in this study, would suggest that gossypol combines with muscle and necrosis occurs. Macrophages phagocytize the debris from these degenerating muscle fibers. Granules of yellowish-brown pigment result from this degeneration. The pigment slowly disappears from the local area. The mechanism by which this occurs is not known.

Histological studies of the liver of chickens fed gossypol would suggest that the pigment reaches the liver through the blood. It passes from the blood into the hepatic sinusoids and is phagocytized by Kupffer cells. The pigment enters the hepatic cells, and it ultimately disappears from them. Some of the pigment apparently leaves the liver by way of the biliary system. Lillie and Bird,[4] in 1950, observed an enlarged gallbladder in chicks fed gossypol and considered this "the only result of toxicity observed in a majority of the cases."

The present study does not contribute much to the chemical mechanism by which gossypol produces necrosis of striated muscle. However, the yellowish-brown, oil red O- and periodic acid-Schiff-positive, and hemosiderin-negative pigment apparently results from the union of gossypol with muscle. In a lower concentration, muscle is injured less by the gossypol. Although some of the injury to the muscle may result from the diluent, the primary effect is due to gossypol. The dilation of the cavities of the pig's heart and the granular appearance of the myocardial fibers, as described recently by Smith,[5] in pigs fed large amounts of gossypol would support the suggestion that gossypol may injure cardiac as well as striated muscle. Gossypol severely injures striated muscle of chickens when given locally in the concentrations used in this experiment. Muscle fibers injured by gossypol become vacuolated and stain positive for

lipids with osmic acid and dark red with the periodic acid-Schiff reagent. Subsequently these injured muscle fibers disintegrate, and the debris is phagocytized by macrophages. It is in the cytoplasm of these phagocytic cells that the characteristic granules of yellowish-brown pigment are best seen. The pigment is rapidly removed from the local area. Some of the pigment while in the liver enters the biliary system.

Menaul,[2] in 1923, observed that gossypol "exerts a hemolytic effect on the erythrocytes." It was his opinion that gossypol inhibits the liberation of oxygen from hemoglobin and reduces the oxygen-carrying capacity of the blood. In our previous study,[6] it was suggested that the hemolytic anemia present in the chickens fed gossypol resulted from the action of gossypol on the erythrocytes. The present study indicates that striated muscle in the chicken is injured by the presence of gossypol. In 1924, Schwartze and Alsberg[3] observed local edema when gossypol was injected subcutaneously. They also noted that gossypol in a concentration of approximately 1:5,000 produced paralysis of the isolated intestines of rabbits within 15 minutes.

The pigment in gossypol-fed chicks (1) is yellowish-brown when stained with hematoxylin and eosin, (2) is partly soluble in alcohol, (3) stains positive with oil red O for fat, (4) stains a dark red with the periodic acid-Schiff reagent, (5) is acid-fast positive, (6) gives a negative Prussian blue reaction for iron, and (7) stains bright red with Mallory's stain for hemofuscin. Ceroid pigment in the liver of rats given *p*-dimethylaminoazobenzene was first described in 1941, by Edwards and White.[8] The characteristics of ceroid pigment, according to Casselman,[9] are (1) a golden-yellow color when unstained, (2) insolubility in organic solvents, (3) sudanophilia, (4) acid-fastness, (5) basophilia, and (6) fluorescence.

Summary

Gossypol obtained from the pigment glands of cottonseed, when injected intramuscularly into chickens, produces necrosis of the muscle. Accompanying this degeneration, there occurs a ceroid-like pigment. This pigment is the same as that resulting from the ingestion of gossypol by chickens. The pigment is phagocytized locally by macrophages. It accumulates in the liver after ingestion, and it slowly disappears. The mechanism of removal is not known; however, some of the pigment enters the biliary tract. It is suggested that the lethal effects produced by gossypol may result from the action of gossypol on vital structures, such as cardiac muscle and erythrocytes.

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Electron Microscopy of Islet Cells in Alloxan-Treated Rabbits

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Introduction

The cytotoxic effects of alloxan on the islets of Langerhans were first discovered by Dunn, Sheehan, and McLetchie,[1] in 1943. Since then numerous investigators [2-10] have described in detail by light microscopy the changes in islets of various species after the administration of alloxan.

Degenerative changes in β-cells of rabbits have been observed as early as five minutes after the administration of alloxan. Bailey et al.[5] reported increased prominence of nuclear membranes and clearing of the cytoplasm with some loss of granules; Hughes et al.[6] observed irregularity in the outline of the β-cells, with small fragments of cytoplasm cast off from the cell surface into pericapillary tissues. The greater resolution and magnification offered by electron microscopy suggested the possibility that changes in the organelles of the β-cells might be detectable during this early period and the site of action of alloxan determined more precisely. The normal ultrastructure of the α- and β-cells of the rabbit has been described by one of us.[11,12]

Materials and Methods

Twenty-two albino rabbits of both sexes weighing 2 to 4 kg. were used. Five of these served as controls. The remaining animals were given injections of 200 mg. per kilogram of a freshly prepared 5% aqueous solution of alloxan* in the marginal ear vein over a period of two minutes. To prevent death from hypoglycemia, those animals surviving 12 hours or longer were given 10-15 cc. of a 10%-15% dextrose solution intravenously and/or subcutaneously every 4 hours for 12 hours, beginning 6 hours after the injection of alloxan.

Three animals died at various intervals and were not used for histologic study. The remaining animals were killed with intravenous pentobarbital (Nembutal) as follows: two each at 5 and 15 minutes, and one each at ½, 1, 1½, 2, 3, 4, 5, 12, and 24 hours and at 6 days after the injection of alloxan. Small pieces of tissue were immediately obtained from the tail of the pancreas and fixed in 1% osmic acid-dichromate fixative buffered to pH 7.2 or 7.6[13] for one hour at room temperature. They were then dehydrated and embedded in methacrylate by the method described previously.[11] Fixation and dehydration times were carefully controlled.

Thick (2μ) and thin sections were cut with glass knives on a Servall-Porter Blum microtome. Islets were localized in thick sections by phase-contrast microscopy; the face of the block was then trimmed with a razor blade to include only the islet and a narrow rim of acinar tissue. Thin sections were examined and photographed with one of three RCA electron microscopes (Models EMU 2C, 2E, and 3B). Electron micrographs were taken at original magnifications of 2,000-6,000 diameters and enlarged photographically as desired.

Additional blocks of pancreas were fixed in Bouin solution, and paraffin sections were cut and stained with aldehyde fuchsin[14] and chrome alum hematoxylin.[15]

Determinations of blood sugar levels were done by the method of Somogyi[16] on samples obtained from marginal ear veins.

Granule counts were done on 20 electron photomicrographs of normal β-cells and 16 electron photomicrographs of β-cells five minutes after alloxan. A planimeter was used to measure the area of β-cell cytoplasm on each photomicrograph and the count expressed as granules per square decimeter of β-cell cytoplasm. All of the counts were corrected to a magnification of 18,000 diameters and the t-test was applied.

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Department of Pathology, Washington University School of Medicine.

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* Eastman Kodak Company.

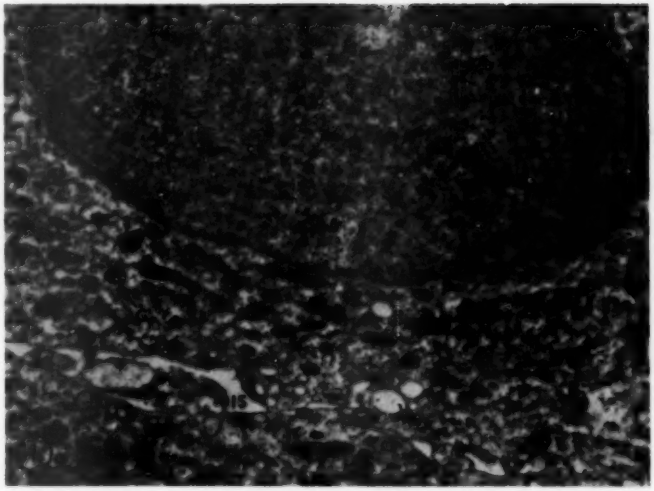

Fig. 1.—Electron micrograph of portions of two normal β-cells. The granules (*G*) are relatively small and few in number. Numerous mitochondria (*M*) are present in the cytoplasm. *IS* indicates intercellular space; *N*, nucleus; reduced 27% from mag. × 20,700.

Observations

The changes which occurred in individual β-cells and islets were variable even within the same animal. However, definite progressive changes were demonstrable during the first 24 hours.

β-Cells.—Five Minutes: In the normal β-cell the granules are small and relatively few in number (Fig. 1). Each granule is surrounded by a distinct membrane. An increase in the number of β-granules, which were distributed uniformly throughout the cytoplasm, was the only change apparent at this time (Fig. 2). This increased granulation was also evident in paraffin sections stained with aldehyde fuchsin.

The granule counts on electron photomicrographs revealed means of $4.9 \pm .68$ † granules per square decimeter for normal β-cells and 36.1 ± 5 † granules per square decimeter for β-cells five minutes after alloxan. The difference in the degree of granulation of the two groups was significant ($P = < 0.001$).

† Standard error of the mean.

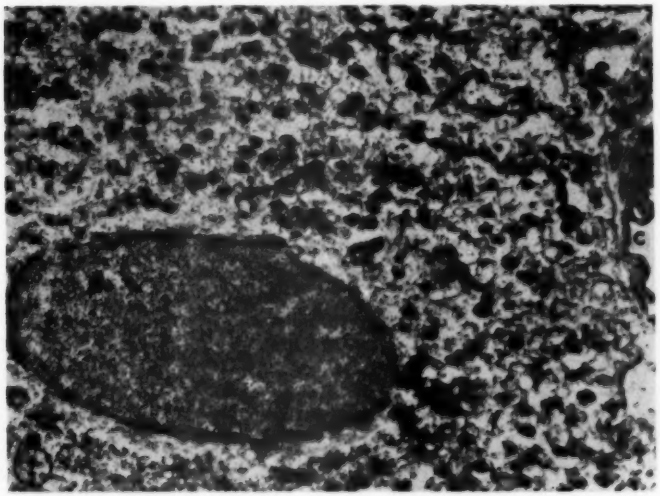

Fig. 2.—Electron micrograph of a portion of a β-cell five minutes after the injection of alloxan. Numerous β-granules (*G*) are scattered throughout the cytoplasm. *C* indicates capillary; *M*, mitochondria; *N*, nucleus; reduced 27% from mag. × 20,700.

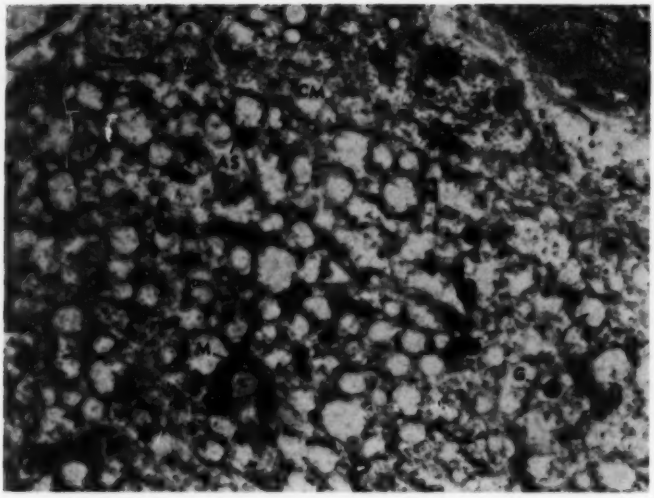

Fig. 3.—Electron micrograph of portions of two β-cells 30 minutes after alloxan. A few distinct β-granules (G) are still present. Some of the numerous vacuoles (V) contain strands of gray amorphous substance (AS). CM indicates cytoplasmic membrane; F, fibrillar material; M, mitochondria; N, nucleus; reduced 27% from mag. $\times$ 20,700.

Fifteen to Thirty Minutes: A definite decrease in the number of distinct β-granules was discernible by electron microscopy. Numerous small vacuoles were apparent throughout the cytoplasm. Some contained distinct granules, while others contained strands of a gray amorphous substance which appeared to be remnants of granules (Fig. 3). These vacuoles probably represent slightly dilated membranous sacs which normally surround the granules.

One to Two Hours: Only occasional distinct granules were seen in the numerous vacuoles which were partially collapsed and beginning to disintegrate. The mitochondria contained vacuoles which were small and scattered at one hour but which became numerous and distended the mitochondrial membranes at two hours (Fig. 4). Remnants of cristae were still discernible in these dilated mitochondria. Intercellular spaces were enlarged, and numerous interruptions in the continuity of plasma membranes had developed. Nuclear membranes were intact. Perinuclear fibrillar material, which has been described in the normal

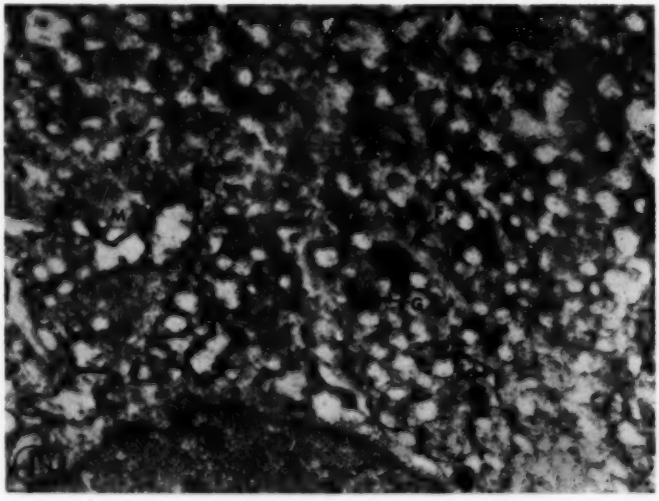

Fig. 4.—Electron micrograph of a portion of a β-cell two hours after alloxan. Very few β-granules (G) are evident. The mitochondria (M) are vacuolated and swollen. F indicates fibrillar material; N, nucleus; reduced 27% from mag. $\times$ 27,600.

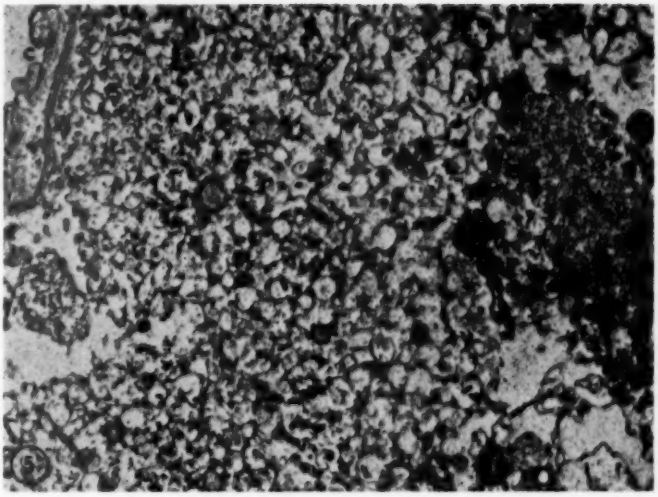

Fig. 5.—Electron micrograph of a portion of a necrotic β-cell five hours after alloxan. The nucleus (*N*) is fragmented. Occasional small swollen mitochondria (*M*) can be identified in the debris. *C* indicates capillary; reduced 27% from mag. × 13,800.

β-cell,[12] remained unchanged (Fig. 4). By light microscopy β-cell granulation) appeared unchanged from that observed at five minutes.

Five Hours: The β-cell cytoplasm was filled with vacuoles. Mitochondria were vacuolated and fragmented (Fig. 5). Nuclear and plasma membranes were fragmented, and their contents had spilled into the intercellular spaces. Distinct β-granules could not be identified with certainty in the debris.

Twelve Hours: Complete destruction of nuclear and plasma membranes had occurred. β-cells had degenerated into masses of vacuolated debris containing small clumped fragmented nuclei (Fig. 6). Vacuolation and fragmentation of mitochondria had progressed to the point where it was impossible to identify them. Macrophages were observed adjacent to capillaries and among the necrotic β-cells (Fig. 6). They were identified on the basis of their irregularly shaped elongated nuclei, numerous

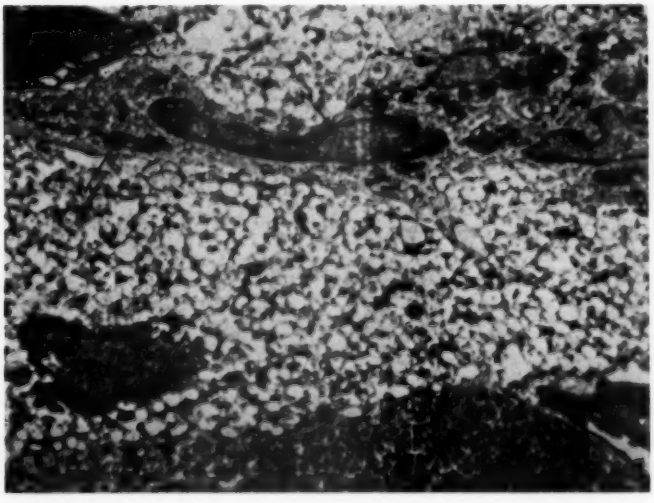

Fig. 6.—Electron micrograph of a portion of a necrotic islet 12 hours after alloxan. A normal α-cell (*AC*) containing numerous granules lies adjacent to a necrotic β-cell with a pyknotic nucleus (*PN*). Adjacent to the capillary (*C*) in the upper left corner lies a macrophage containing foci (*F*) of ingested necrotic debris. *N* indicates nucleus; *M*, mitochondria; reduced 27% from mag. × 13,800.

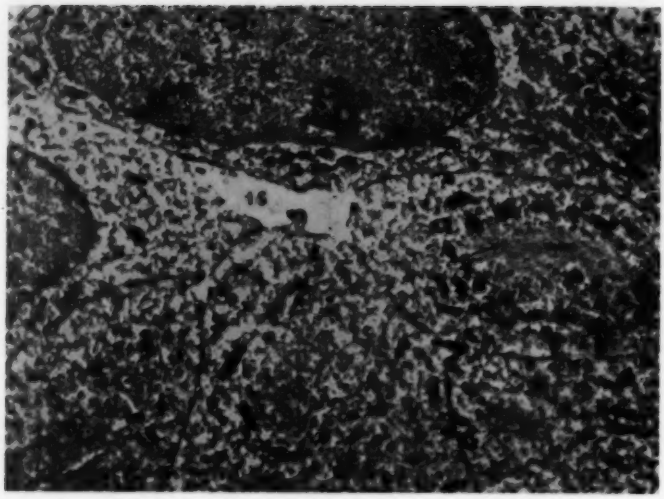

Fig. 7.—Electron micrograph of a portion of an islet 24 hours after alloxan. Distinct fibrillar material (*F*) is present in one β-cell. Distinct β-granules are absent. *IS* indicates intercullular space; *M*, mitochondria; *N*, nucleus; reduced 27% from mag. × 13,800.

pseudopodia, and foci of ingested necrotic debris within their cytoplasm.

Twenty-Four Hours: Necrotic β-cells and macrophages containing debris were apparent in most islets. A few small islets were found in which the β-cells were degranulated (Fig. 7). They were identified as β-cells on the basis of perinuclear fibrillar material which was observed in some of them. A few of these degranulated but otherwise intact β-cells were observed in islets which also contained frankly necrotic β-cells.

Six Days: Severe hyperglycemia (360 mg. %) and glycosuria were present in this animal at the time of death. The islets, which were small and difficult to find by phase microscopy, were composed of normal appearing α-cells and degranulated β-cells containing perinuclear fibrillar material (Fig. 8). β-granules were not observed by light microscopy in paraffin sections stained with aldehyde fuchsin.

It was not possible to determine whether changes occurred in the golgi apparatus because of the early marked vacuolation in

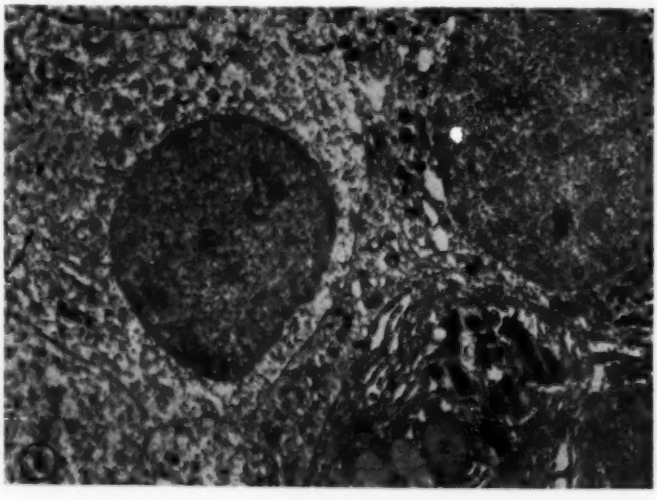

Fig. 8.—Electron micrograph of a portion of an islet and two acinar cells six days after alloxan. Distinct granules (*G*) are present in the α-cell in the upper right corner. In the lower right corner zymogen granules (*Z*) and lipid droplets (*L*) are seen in a portion of an acinar cell. A circular area of fibrillar material (*F*) is discernible in one of the β-cells. *ER* indicates ergastoplasm; N, nucleus; reduced 27% from mag. × 13,800.

the cytoplasm. Abnormalities were not demonstrable in the capillary endothelium of the islets.

α-Cells.—The ultrastructure of the α-cells appeared unchanged throughout the period of observation. A normal α-cell adjacent to necrotic β-cells is illustrated in Figure 6.

Acinar Cells.—Lipid droplets were observed in pancreatic acinar cells at 24 hours and were numerous at 6 days. Ergastoplasm, zymogen granules, and mitochondria of acinar cells appeared normal in all the animals.

Comment

Five minutes after injection of alloxan an increase in the number of β-cell granules was evident by both light and electron microscopy and was substantiated by granule counts on electron photomicrographs. This observation is in agreement with that of House et al.,[9] who found that β-granule concentration reached a peak 40 minutes after the administration of alloxan in the hamster. They suggested that alloxan produces an initial stimulation of β-cells, since increased cytoplasmic basophilia and increased vascular engorgement of the islets were also observed. Hypergranulation could be produced by an increased rate of granule formation, a decreased rate of granule breakdown, or an increase in the rate of both processes with a disproportionate increase in the rate of formation. Shipley and Beyer,[17] House et al.,[9] and others have observed an initial hypoglycemia in this period. This finding suggests either an increase in circulating insulin or a potentiation of the effects of circulating insulin by alloxan. Further electron microscopic studies on the acute effect of large amounts of glucose and of insulin may help elucidate whether the transient hypergranulation followed by vacuolation and disintegration of the granules represents β-cell stimulation or inhibition of granule breakdown. Regardless of the mechanism involved, the observation that distinct β-granules can be formed within a period of five minutes and disintegrate quickly in the ensuing intervals suggests that β-cells can respond rapidly to stimulation and that the half-life of β-granules under these conditions may be very short. Use of fluorescent antibody techniques for the demonstration of insulin [18] should aid in determining whether hypergranulation represents an increase in the insulin content of the cell.

By electron microscopy normal β-granules are seen to be contained within sacs which are not evident by light microscopy. Two steps appear to be involved in the disintegration of granules after alloxan. Within 30 minutes after injection most of the granules have "dissolved," but their amorphous remnants are contained within the sacs which are still intact but slightly dilated. By two hours the sacs have partially collapsed and begun to disintegrate. During this time there is no evidence of degranulation seen by light microscopy. This observation suggests that the amorphous granule remnants have retained a strong affinity for aldehyde fuchsin.

The first definite cytologic evidence of degenerative change was swelling of the mitochondria, which had become severe at two hours. Brachet [21] has stated that the mitochondria in an injured cell first lose their delicate internal structure and then swell and tend to become spherical. Further degenerative changes, consisting of fragmentation of nuclear and plasma membranes, were seen later.

The presence of macrophages in islets after alloxan has been demonstrated by Ruangsiri [22] in living mice and in stained sections. By electron microscopy they were seen to be numerous at 12 and 24 hours after alloxan and to have ingested debris.

The degranulated β-cells observed 24 hours and 6 days after alloxan may represent newly formed cells, as the result of regeneration, or cells which escaped the effects of alloxan. Whether the absence of specific granules in these cells represents a hyperactive state, with an equilibrium between the rate of formation and release

of secretory products, or an inactive state is not known.

The significance of perinuclear fibrillar material in β-cells remain unexplained, since it did not undergo any specific changes. Absence of degenerative changes in the ultrastructure of the α-cells corroborates the specificity of the cytotoxic action of alloxan on β-cells.

The origin and significance of lipid droplets observed in the acinar cells at 24 hours and 6 days is unknown at the present time. They did not appear to originate from organelles, since no degenerative changes were observed in the latter. Their development did not appear to be associated with either hyperglycemia or gross hyperlipemia. Occasional lipid droplets have been observed in acinar cells of normal guinea pigs [21]; numerous lipid droplets have been observed in the guinea pig after treatment with cobalt.[22]

Summary

The cytotoxic effects of alloxan on the ultrastructure of the rabbit pancreas were studied. Increased β-cell granulation was evident five minutes after alloxan by both light and electron microscopy. This increased granulation was followed in 15-30 minutes by a simultaneous decrease in the number of distinct β-granules and the appearance of vacuoles containing remnants of granules. Degenerative changes were observed in the mitochondria at one to two hours. By five hours the mitochondria and the nuclear and plasma membranes were fragmented. Macrophages had engulfed necrotic debris at 12 and 24 hours. A few small islets consisting of degranulated β-cells were found at 24 hours. Occasional degranulated β-cells were also seen in islets containing necrotic β-cells. At six days the islets were small and were composed of only a few degranulated β-cells and normal-appearing α-cells. Lipid droplets were observed in the pancreatic acinar cells at 24 hours and were numerous at 6 days.

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Tumoral Amyloidosis of the Lungs

A Case Report

MARTIN DUKE, M.D., Boston

Introduction

Amyloid deposition in the lungs as a diffuse infiltration within alveolar septa has been described often. Its presence in the form of large tumor-like deposits within the lung parenchyma is of much rarer occurrence. The following report will describe the clinical and pathological features of such a case, together with a brief review of the pertinent literature.

Report of Case

An 87-year-old white man was admitted to the hospital with the chief complaint of progressive shortness of breath over a three-week period, becoming severer two days prior to admission and associated with swelling of the ankles. The patient stated that he had had a cough for the previous 15 years, with hemoptysis and at times with frank hemorrhage. The cough had become worse a few days prior to hospitalization. Fever, chills, orthopnea, and paroxysmal nocturnal dyspnea were denied.

A review of the past history revealed that the patient had been hospitalized on only one previous occasion. This occurred six years prior to this admission for a traumatic fracture of the left humerus. At that time an x-ray of the chest was reported as showing a slight generalized degree of emphysema. A concomitant electrocardiogram revealed complete auriculoventricular heart block, with findings indicative of myocardial disease. The only medication the patient had previously used was sulfonamide tablets. His occupation had been that of a newspaper writer.

Physical examination revealed an elderly male with cyanosis, dyspnea, and a cough productive of blood-tinged sputum. The temperature was 101.6 F; pulse, 125 per minute; respiratory rate, 28 per minute, and blood pressure, 150/100 mm. Hg. Pertinent physical findings included flat neck veins in an erect position, the point of maximal cardiac impulse at the left sixth intercostal space in the midclavicular line, distant heart sounds, A_2 equal to P_2, and an increase in the anteroposterior diameter of the chest, with dullness to percussion, decreased breath sounds, and medium-to-coarse moist rales at both lung bases. The liver descended slightly below the right costal margin and was tender. The legs showed 3+ to 4+ pitting edema, with cyanosis of the nail beds. No clubbing was observed.

Laboratory studies were as follows: a negative urinalysis aside from 1+ albuminuria; hemoglobin 16.0 gm. per 100 cc.; white blood cell count 14,000 per cubic millimeter, with a slight shift to the left; sedimentation rate 38 mm. in one hour. Two sputum smears for acid-fast bacilli were negative.

Treatment was started with tetracyline (Achromycin), sulfisoxazole (Gantrisin), and meralluride (Mercuhydrin). However, the patient remained febrile, and when the blood pressure fell shortly after admission, it was necessary to place him in an oxygen tent and start levarterenol ([*l*-norepinephrine] Levophed) infusions. An electrocardiogram at that time revealed auricular fibrillation, numerous ventricular premature contractions, and a left bundle-branch block. On the sixth hospital day the patient died.

A postmortem examination was performed seven hours after death. The body was that of a white man, 5 ft. 5 in. (165 cm.) tall and weighing 60 kg. External examination was unremarkable.

The heart appeared large, weighing 530 gm., with no undue thickness of the walls of any chamber. An area of fibrosis was present in the posterior region of the interventricular septum near the apex. The coronary arteries contained severe atherosclerotic changes, with considerable narrowing of their lumina. There was a marked amount of calcification of the aortic valves, involving all but the free edges of the cusps and causing a moderate degree of stenosis.

The left lung weighed 640 gm. and the right lung 720 gm. Several nodules protruded approximately 0.5 cm. above the surfaces of the right middle lobe and both lower lobes. These nodules, as well as many others observed on sectioning

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Fig. 1.—Lung with large multiple tumor-like masses of amyloid at the base.

the lungs, were round or polygonal in shape and varied in diameter from 3-5 cm. (Fig. 1). When these masses were themselves sectioned, they appeared a translucent gray-to-yellow color and lardaceous in consistency, with hard areas within them. For the most part they were located outside the bronchi, even though in areas they appeared partially to surround these structures. The masses were very discrete, with sharp borders, except in some places where their peripheral edges appeared to fade out into the surrounding parenchyma. On application of strong iodine (Lugol's) solution, they stained a dark mahogany brown. The trachea and bronchi were unremarkable.

Other findings included a severe degree of generalized arteriosclerosis, a calculus in the gallbladder, several small polyps in the large intestine, and diverticuli in the sigmoid colon. All other organs were grossly normal.

The microscopic examination showed the following: Sections from the tumor-like masses of the lungs revealed sheets of uniform eosinophilic-staining material (hematoxylin and eosin) having a flaky appearance in some areas and having the form of parallel or concentric layers in other regions (Fig. 2). This material stained red with the Congo red stain, metachromatic with methyl violet, a khaki color with Van Gieson's stain, blue-purple with Masson's trichrome stain, and brown with phosphotungstic acid-hematoxylin stain, and it showed a pale reaction with periodic acid-Schiff stain. With each of these stains the intensity of the reaction varied from area to area. These findings, as well as the gross color reaction to iodine, confirmed the identity of this material as amyloid.

Within the substance of the amyloid masses there was cuboidal metaplasia of alveoli in those few areas where the latter could still be recognized. Scattered multinucleated giant cells were present, some of which appeared to be engulfing the amyloid.

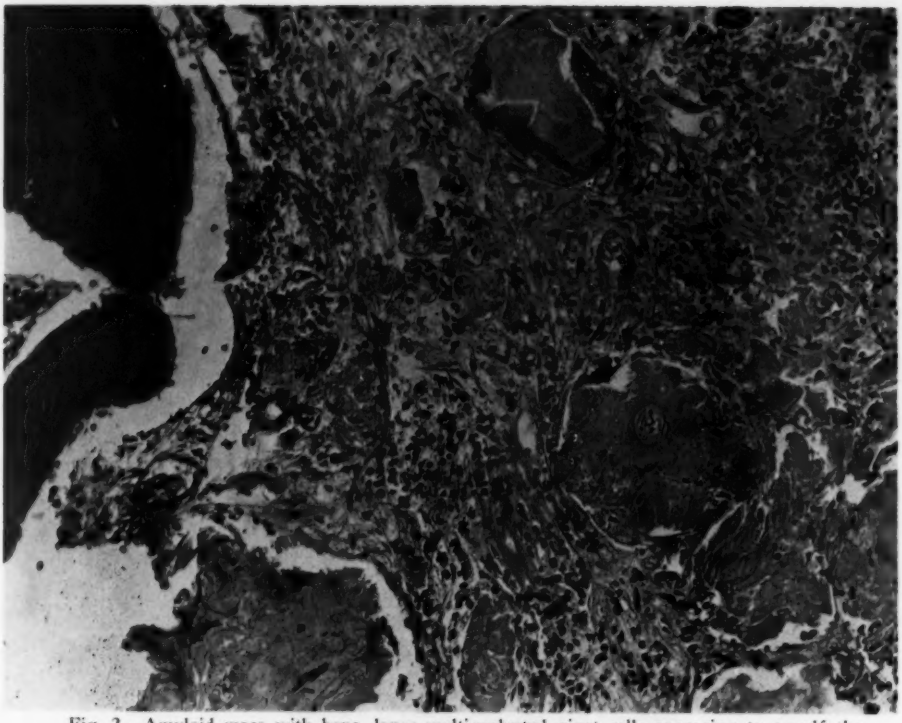
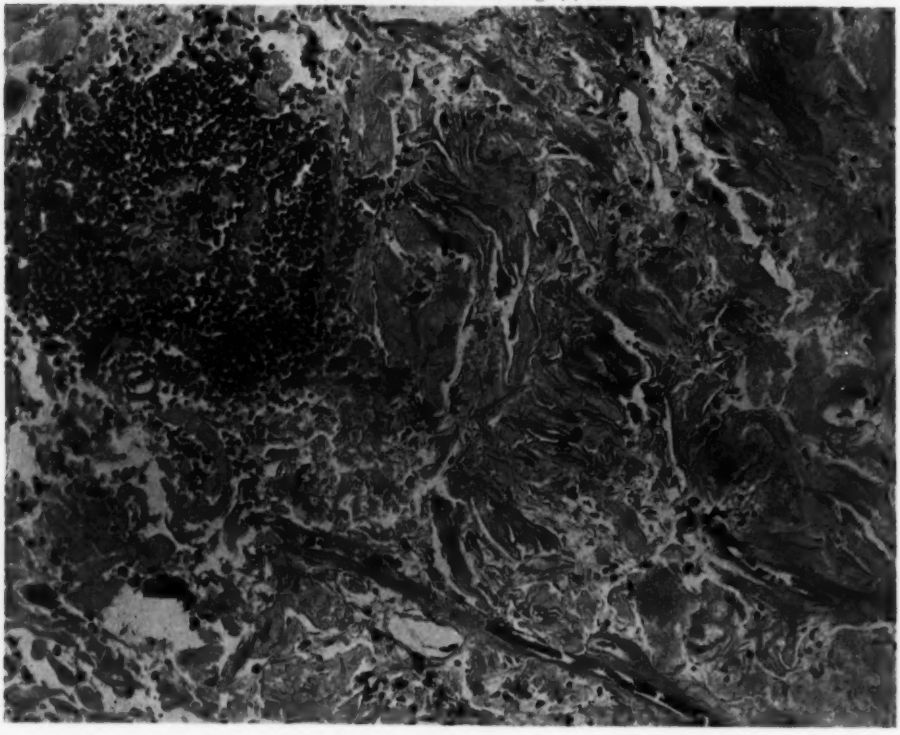


Fig. 2.—Amyloid mass with bone, large multinucleated giant cells appearing to engulf the amyloid, and scattered lymphocytes and plasma cells; reduced 40% from mag. × 400.

Fig. 3.—Amyloid with a focal area of lymphocytes and plasma cells containing homogeneous eosinophilic material in its midst; reduced 40% from mag. × 400.



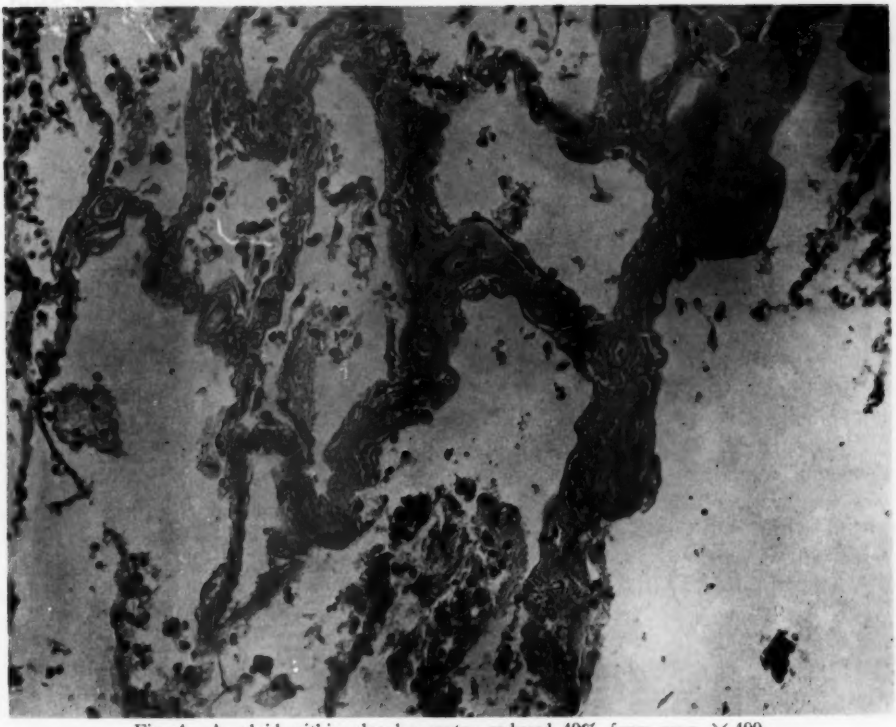
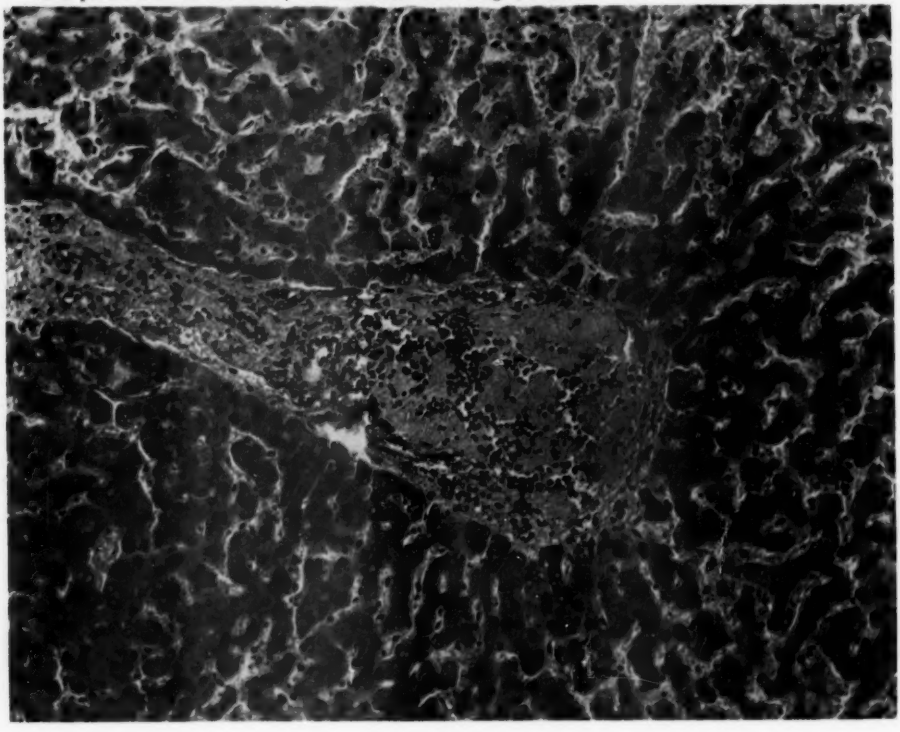


Fig. 4.—Amyloid within alveolar septa; reduced 40% from mag. × 400.

Fig. 5.—Homogeneous eosinophilic material associated with lymphocytes and plasma cells in a portal area of the liver; reduced 40% from mag. × 400.

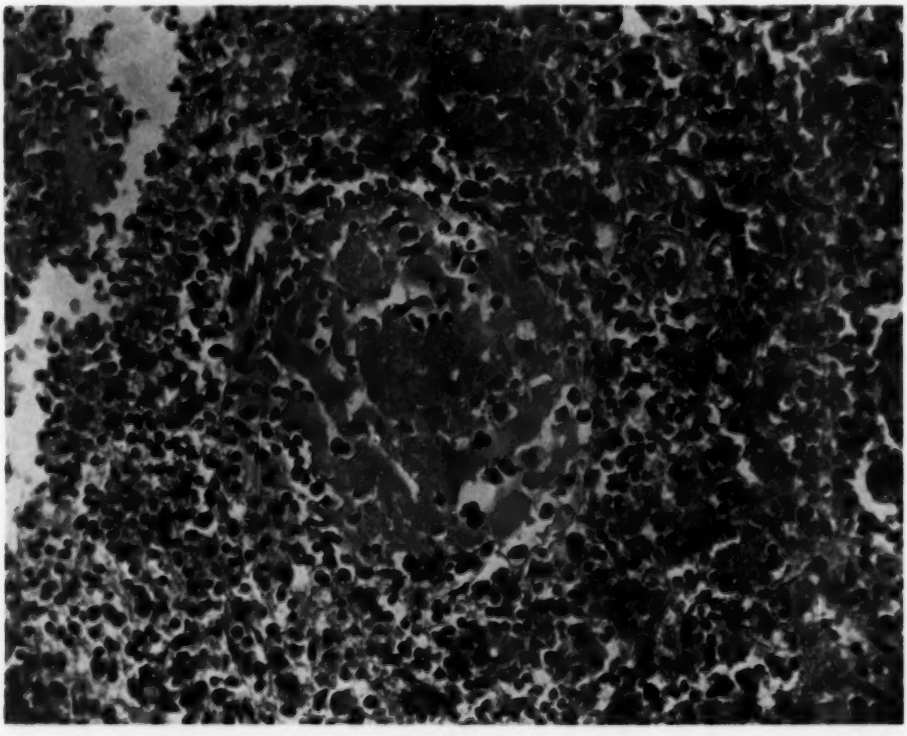


Bone was seen containing a cellular marrow. Groups of lymphocytes and plasma cells were present within the amyloid nodules as well as at their periphery. They contained amongst them a uniform-appearing eosinophilic material which looked like amyloid but did not have its "typical" staining properties (Fig. 3). Scattered in areas of the lungs beyond the gross limits of the amyloid masses were other groups of lymphocytes, plasma cells, and occasional multinucleated giant cells, associated with similar material which again stained negative for amyloid. Other sections of lung revealed a few alveolar septa which were thickened by deposits of "typical"-staining amyloid (Fig. 4). An organizing lobular pneumonia was present.

In some of the portal areas of the liver groups of lymphocytes and plasma cells surrounded homogeneous eosinophilic material which did not stain characteristically for amyloid (Fig. 5). Within the lymphoid follicles of the spleen deposits of similar appearing material were present, at times appearing to extend to and surround small arteries and arterioles (Fig. 6). However, in other areas of the spleen, other small arteries were seen which did contain deposits of "typical"-staining amyloid within their walls.

Sections of mediastinal lymph nodes revealed granulomatous lesions with epithelioid cells, multinucleated giant cells, and homogeneous eosinophilic material (Fig. 7). No "typical"-staining amyloid could be demonstrated, and no foreign material or necrosis was apparent. Other areas of the nodes revealed hyalinized scar tissue containing a few doubly refractile crystalline bodies. In the heart there was fibrous tissue replacement of myocardial fibers. An oc-

Fig. 6.—Lymphoid follicle in the spleen with eosinophilic material resembling amyloid; reduced 40% from mag. × 960.



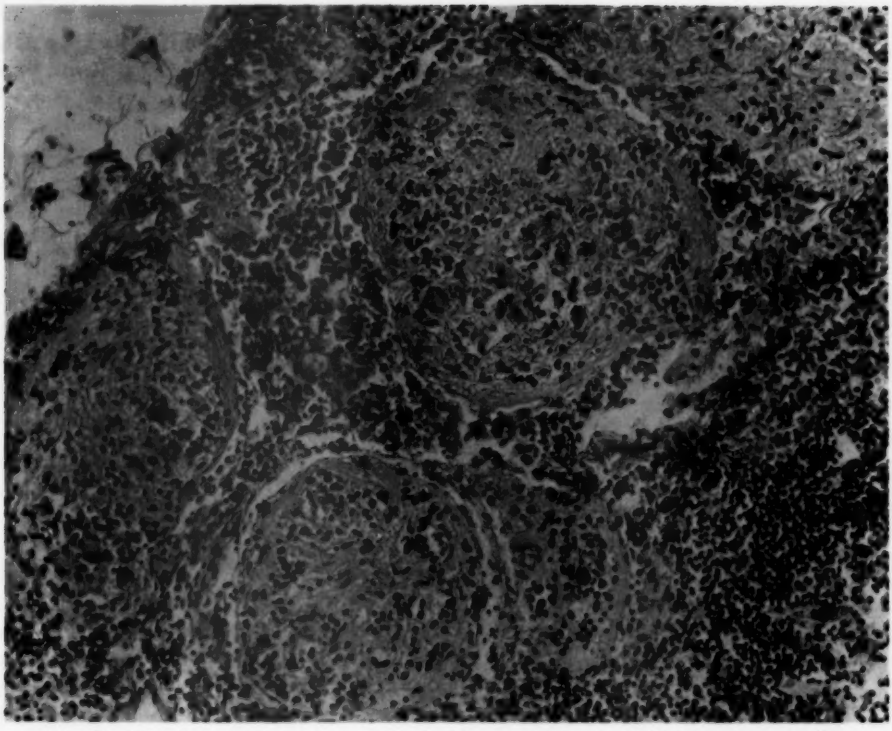


Fig. 7.—Granulomatous lesions in a mediastinal lymph node; reduced 40% from mag. × 400.

casional small artery in the kidneys and testes contained scant amounts of amyloid positive-staining material. Sections of bone revealed a somewhat hyperplastic marrow with no evidence of an increase in plasma cells.

Summary of pathological findings: Tumor-like deposits of amyloid in the lungs; scattered "typical" and "atypical" amyloid deposits in the lungs, liver, spleen, lymph nodes, kidneys, and testes; granulomatous lesions of unknown etiology in mediastinal lymph nodes; arteriosclerotic heart disease with an old healed myocardial infarct; calcific aortic stenosis; generalized arteriosclerosis, severe; organizing lobular pneumonia; cholelithiasis; diverticulosis of the large intestine; polyps of the large intestine.

Classification

Symmers [1] has reviewed many of the classifications of amyloid disease reported in the literature and has himself classified amyloidosis on a clinicopathologic basis.

1. Generalized amyloidosis associated with a recognized predisposing disease (generalized secondary amyloidosis)

2. Generalized amyloidosis in the absence of a recognized predisposing disease (generalized primary amyloidosis)

3. Localized amyloidosis

Lunzenauer [2] has referred specifically to amyloid tumors in his classification.

1. Amyloid tumor with typical or atypical distribution
   (*a*) With a basic sickness
   (*b*) Without a basic sickness

2. Amyloid tumors, parts of which are tumor forming and parts of which are a diffuse infiltration into the same organ system
   (*a*) With a basic sickness
   (*b*) Without a basic sickness

3. Isolated or local amyloid tumors
   (*a*) With a basic sickness
   (*b*) Without a basic sickness

The terms primary and secondary appear to have significance only when referring to the absence or presence of a demonstrable or associated disease. It has even been suggested that all varieties of amyloid disease are related manifestations of one fundamental disturbance.[1]

Pathology

Descriptions of the pathology of amyloid involvement of the lungs have been presented by many authors.[1,3-5] The form and even the distribution of amyloid deposition may be the same regardless of whether it be of the primary or secondary type. It may be deposited in the walls of the medium or small blood vessels, in the alveolar septa as a diffuse process, in the lung parenchyma as isolated or multiple masses, in the walls of the trachea or bronchi, or in various combinations of these. The process may be a relatively minor one with minimal or no clinical findings as in this case, or there may be major pathological changes with marked symptomatology. The latter circumstance can manifest itself with symptoms due either to narrowing or obstruction of the respiratory passages[3,4,6] or to cor pulmonale.[7]

In a recent review of the literature, Glauser[5] reported on only 10-12 cases of tumor-like masses of amyloid in the lung. It should be noted, however, that this review includes not only cases of the lung parenchyma but also those involving the lower respiratory tract to the exclusion of the more peripheral lung fields except by direct extension. It seems reasonable, therefore, that, since the lung lesions can occur without tracheal and major bronchial involvement, a further clinicopathological differentiation would be in order, if for no other purpose than to become aware of and recognize this entity. The bilateral lesions, their multiplicity, their location within the lung parenchyma, and the different (and often all too sparse) clinical findings in contrast to cases involving primarily the trachea and major bronchi would appear to justify this. Thus, of the 14 cases of lower respiratory tract involvement reviewed by Schottenfeld and his associates,[8] only those of Herxheimer,[8] Meyer,[9] and Hallerman[10] would appear to be similar to the case reported in this presentation. To these may be added another reported by Glauser,[5] even though it also differed somewhat owing to the presence of associated involvement of the upper respiratory tract.

The nodular lesions in the lungs are described grossly as being of hard consistency, having a waxy yellow-to-gray translucent appearance,[4,5,11] and varying in size up to 6 cm.[12] Microscopically they consist of uniform eosinophilic staining masses (hematoxylin and eosin stain), often having the flaky appearance described by Stark and McDonald.[13] There is usually complete loss of the original lung structures, even though blood vessels can be found coursing through the masses,[5] as well as a few recognizable alveoli with cuboidal metaplasia of their lining cells.[11] The presence of giant cells is a not uncommon accompaniment of amyloid in the lung as well as in other locations.[5,11,12] Bone has been noted in many cases,[3,5] and it is stated by Glauser[5] that in tumor-like amyloid of the lung its finding is almost the rule. The presence of plasma cells and lymphocytes, found in small numbers in association with the amyloid in this case, has been interpreted with various degrees of significance by several authors.[5,14]

It is generally agreed that amyloid is not necessarily of constant chemical composition, both in the same case and in different cases. Amyloid consists of a protein or variety of proteins associated with a polysaccharide moiety. Its different components account for its staining characteristics, and its variable composition is the reason for the inconstancy of these staining properties. Symmers[1] has reviewed this subject.

Etiology

On the basis of the clinical and autopsy findings, no specific etiology can be cited as

the cause for the unusual form and distribution of amyloid in this case. Whether a local lesion of inflammatory or neoplastic origin could be the precipitating cause for the amyloid deposition is speculative. Abnormalities in protein metabolism,[15] an increased activity of the reticuloendothelial system,[16] and the presence of plasma cells [5] have all been related either experimentally or with autopsy material to amyloid deposition. Cases of multiple myeloma and localized plasmacytoma [17,18] associated with amyloid are well known. Whether multiple extramedullary plasmacytomas or scattered accumulations of plasma cells could cause the formation of amyloid in the lungs, as seen in this case, prior to being obliterated by these deposits is a matter of conjecture.

It is suggested that an awareness of this disease entity, based upon the clinical features, x-ray examinations, laboratory studies such as electrophoretic differentiation of plasma proteins, and biopsy material with fluorescent-antibody studies, may be of considerable aid not only in establishing the diagnosis and the cause of tumoral amyloidosis of the lungs but also of other varieties of amyloid deposition.

Summary

The clinical and autopsy findings of a case of tumoral amyloidosis of the lungs are presented. The classifications, pathology, and etiology of amyloid disease as they pertain to this case are briefly reviewed.

The photographs of the microscopic slides were prepared by Mr. George Ozaki.

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News and Comment

Van Meter Prize Award.—The American Goiter Association again offers the Van Meter Prize Award of $300 and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The award will be made at the annual meeting of the Association, which will be held in the Drake Hotel, Chicago, April 30 and May 1 and 2, 1959.

The competing essays may cover either clinical or research investigations, should not exceed 3,000 words in length, and must be presented in English. Duplicate typewritten copies, double spaced, should be sent to the Secretary, Dr. John C. McClintock, 149½ Washington Ave., Albany 10, New York, not later than Jan. 15, 1959. The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the winning essay by the author if it is possible for him to attend.

Course in Advanced Oral Pathology.—The second annual course in Advanced Oral Pathology will be given by the University of Minnesota during the week of April 20-24, 1959. The Course consists of both lectures and laboratory. The subjects and faculty are as follows: Tumors of the Skin—Dr. Herbert Z. Lund, Professor of Dermatology, University of North Carolina; Tumors of Soft Tissues—Dr. Raffaele Lattes, Professor of Surgical Pathology, Columbia University; Skin Dermatoses—Dr. Robert Goltz, Associate Professor of Dermatology, University of Minnesota; Non-Neoplastic Diseases of the Jaws—Dr. William Shafer, Professor of Oral Pathology, Indiana University; Tumors of Bone—Dr. David C. Dahlin, Associate Professor of Pathology, Mayo Clinic; Radiation Pathology—Dr. Donn Mosser, Professor of Radiology, University of Minnesota; Advanced Roentgenographic Diagnosis—Dr. Edward Stafne, Professor of Dentistry, Mayo Clinic; Salivary Gland Pathology—Dr. Anand Chaudhry, Associate Professor of Oral Pathology, University of Minnesota; Odontogenic Lesions—Dr. Robert Gorlin, Professor of Oral Pathology, University of Minnesota.

The tuition will be $65. The course registration is limited to 15 persons. Housing for the five days will be approximately $15. Interested persons should immediately contact Dr. Robert J. Gorlin, Chairman, Division of Oral Pathology, School of Dentistry, 242 Owre Hall, University of Minnesota, Minneapolis 14, Minn.

Fourth International Congress for Clinical Pathology.—The Fourth International Congress for Clinical Pathology will be held in Madrid from June 13 to 17, 1960. Further information may be obtained from the Secretary of the Organizing Committee, Dr. José Aparicio Garrido, Faculdad de Medicina, Parabellon No. 2, Ciudad Universitaria, Madrid.

Training Program in Experimental Pathology.—The Department of Pathology of The Mount Sinai Hospital, New York, has been awarded $171,000 by the United States Public Health Service as a research training grant for experimental pathology for five years. Under the program, training of residents and research fellows (trainees) in experimental pathology will be integrated with that of anatomical and clinical pathology. The grant also permits the appointment of a coordinator for the organization of the training in various techniques of experimental pathology.

DEATHS

Dr. Edwin S. Gault.—Dr. Edwin S. Gault, Professor of Pathology, Temple University School of Medicine, died on Sept. 1, 1958.

Dr. Nathan Chandler Foot.—Dr. Nathan Chandler Foot, Professor Emeritus, Cornell University Medical College, died on Sept. 5, 1958.

PERSONAL

Dr. James R. Cash Goes to Pakistan.—Dr. James R. Cash, Professor of Pathology, University of Virginia, is spending the next two years in Karachi, Pakistan, as Professor of Pathology in the Basic Medical Science Institute. The institute was founded by the Pakistani government, the International Co-operation Administration of the U. S. State Department, and Indiana University, and is to function in the training of graduates of Pakistan medical schools for service in the medical schools of that country.

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